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INTERACTIONS OF *TYLENCHULUS SEMIPENETRANS* INFECTION, SOIL SALINITY, AND CITRUS ROOTSTOCKS

By

WILLIAM PHATU MASHELA

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For all the South African Einsteins who are being denied
an opportunity to discover relativity.

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By

William Phatu Mashela

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Chairperson: Dr. L. W. Duncan
Cochairperson: Dr. R. McSorley
Major Department: Entomology and Nematology

The nematode *Tylenchulus semipenetrans* and salinity each can reduce citrus growth and yield. No commercially used citrus rootstock is both tolerant to salinity and resistant to *T. semipenetrans*. Thus, interactions of salinity and *T. semipenetrans* were studied using a wide range of citrus rootstock germplasm. Results are discussed relative to untreated controls.

Cyclic salinity, common in citrus-producing regions with wet and dry seasons, increased *T. semipenetrans* densities on three soil types. Also, cyclic salinity reduced host plant resistance when expressed as nematode female development and egg production.

Tylenchulus semipenetrans reduced salt tolerance in citrus rootstocks representing a wide range of salt tolerance, under both greenhouse and field conditions. The nematode

consistently increased foliar Cl and Na; whereas it reduced foliar K along with K, Cl, and Na in roots. Also, infected roots had high levels of starch.

Tylenchulus semipenetrans may alter the partitioning of osmoticum ions (Cl, Na, K) by increasing concentrations of nonstructural carbohydrates in roots. This hypothesis was tested by inducing high nonstructural carbohydrates in roots through mechanical root pruning, which simulated nematode effects on Cl, K, and Na. Also, nematodes and pruning each reduced osmotic potential in seedlings. Thus, the efflux of osmoticum ions counteracts the assimilate-reduced osmotic potential in root cells when increased levels of assimilates are shunted belowground.

When nematodes and salinity were separated in seedlings with split-roots, nematode densities were higher than when nematodes were alone. Thus, salinity effects on nematodes were systemic through the plant. However, nematode effects on Cl and Na accumulation in leaves were nonsystemic.

Salinity exacerbated the deleterious effects of nematodes on citrus. Therefore, management of *T. semipenetrans* becomes more critical as soil salinity increases.

CHAPTER 1
INTRODUCTION

Worldwide, salinity concerns in agricultural production are increasing (Carter, 1975; Epstein et al., 1980; Nabors, 1984). These concerns can no longer be looked upon in a traditional sense of associating salinity with semi-arid and arid regions (Bohn et al., 1984). The scarcity of high quality water during dry seasons in humid zones causes growers to use poor quality water (Carpena et al., 1969; Peynado and Young, 1969; Syvertsen et al., 1989), including municipal wastewater, which is inherently saline (Koo and Zekri, 1989). Thus, widespread use of more saline water for supplemental irrigation during dry seasons extends concerns about salinity to large agricultural regions.

Seawater intrusion is the major contaminant of good quality water along coastal regions (Graham, 1990), such that salt concentrations in some wells in the coastal areas of the United States (Graham, 1990; Parker, 1945; Reichenbaugh, 1972; Stringfield, 1930; Wander and Reitz, 1950; Young and Jamison, 1944) and Israel (Bielorai et al., 1988) are rising. For instance, the chloride (Cl) content of the main coastal aquifer in Israel, a major source for citrus irrigation, increases at the rate of 2 mols Cl/m^3 H_2O per year (Bielorai

et al., 1988). Salinity also can be an inland problem of South Africa (Cohn, 1976), Spain (Carpena et al., 1969; Nieves et al., 1991) and Texas (Peynado and Young, 1969), particularly during the dry seasons.

Machmer (1958) demonstrated that salinity could enhance the population densities of the citrus nematode, Tylenchulus semipenetrans Cobb. In South Africa the highest densities of T. semipenetrans occur in areas with high salinity (Cohn, 1976). Higher population levels of this parasite in Israel also occur in the relatively saline coastal areas and in the Negev desert (Cohn et al., 1965). However, salinity suppressed juvenile eclosion of this and other nematode species in fallow soil (Dropkin et al., 1958). Also, an osmotic potential of -1.01 MPa reduced motility of T. semipenetrans juveniles; whereas -4.05 MPa completely restricted motility (Viglierchio et al., 1969). Conditions whereby salinity enhances population densities of this nematode have not been resolved.

Tylenchulus semipenetrans induces slow decline and replant disorders of citrus (Cobb, 1914; Thomas, 1913). Slow decline symptoms are severe under additional salinity stress (O'Bannon and Esser, 1985). On the east coast of Florida, where salinity occurs along with poorly drained soils, T. semipenetrans can induce severe slow decline symptoms. In contrast, in central Florida, with good quality irrigation water and deep well-drained sandy soils, citrus trees infected

with high nematode densities may have few or no decline symptoms (O'Bannon and Esser, 1985). Similar contrasts are prevalent in South Africa (Cohn, 1976) and Arizona (Reynolds and O'Bannon, 1963). Also, the clinical symptoms of slow decline are similar to those associated with chronic salinity stress and nutrient deficiency (Greenway and Munns, 1980; Levitt, 1980) and include: smaller leaf and fruit, leaf chlorosis, sparse foliage, die-back of young twigs, and an overall decline in tree vigor.

Tylenchulus semipenetrans infection of roots decreased K in citrus leaves (Fouche et al., 1977; Milne and Willers, 1979; Van Gundy and Martin, 1961) and roots (Labanauskas et al., 1965). Salinity also reduced K in citrus leaves (Alva and Syvertsen, 1991; Behboudin et al., 1986; Cooper, 1961) and in roots (Behboudin et al., 1986). The parasite also increased Na in citrus leaves when interacting with both high pH and high soil K (Van Gundy and Martin, 1961). However, the effects of this nematode on salt tolerance have not been studied.

Salt tolerance in citrus has been defined as the ability of roots to exclude excess Cl and (or) Na from shoots (Cooper, 1961; Maas, 1993). Overall, citrus is relatively more sensitive to salinity (Maas, 1993; Shalhevet and Levey, 1990) than other plant species. Two commercial citrus rootstocks, Cleopatra mandarin (*Citrus reticulata* Blanco) and Rangpur lime (*C. reticulata* var. austera Swingle), have limited salt

tolerance relative to other citrus species (Maas, 1993). However, there is no commercial citrus rootstock that is both salt tolerant and resistant to T. semipenetrans (Newcomb, 1978).

Since salinity in irrigation water is increasing, and since the highest population densities of T. semipenetrans occur in regions with high salinity, it seems important to study the effects of salinity on nematode resistance and conversely, the effects of T. semipenetrans on salt tolerance. The specific objectives of this research were: (1) to evaluate conditions under which salinity increases population densities of T. semipenetrans, (2) to determine whether salinity affects resistance to T. semipenetrans in citrus rootstock seedlings representing a wide range of nematode resistant germplasm, (3) to determine the effects of T. semipenetrans on salt tolerance in citrus rootstock seedlings representing a wide range of salt tolerant germplasm, and finally, (4) to investigate potential mechanisms by which T. semipenetrans affects salt tolerance in citrus. Mechanistic studies will focus on split-root systems to separate nematode and salinity treatments within the same plant and root pruning treatments. Plant responses will include the partitioning of osmotically active ions and nonstructural carbohydrates, growth, osmotic potential, transpiration, and CO_2 assimilation.

CHAPTER 2
REVIEW OF LITERATURE

Introduction

The major objective of this research was to study the reciprocal interactions of the citrus nematode, _Tylenchulus semipenetrans_ Cobb, and NaCl amended irrigation water on the subtribe Citrinae. The nonstructural carbohydrate status of plants was also investigated because cell concentrations of nonstructural carbohydrates appear to be intimately associated with the accumulation and the partitioning of nutrient ions in plants (Rodney et al., 1956). _Tylenchulus semipenetrans_ parasitism of root previously reduced osmotically active ions in roots (Labanauskas et al., 1965), but its effects on carbohydrates have not been resolved (Hamid et al., 1985). Because root pruning may increase starch in the remaining roots, may be an important tool in enhancing the characterization of role of nonstructural carbohydrates in the allocation of ions in roots and leaves.

Tylenchulus semipenetrans

Management of _T. semipenetrans_-induced slow decline and replant diseases of citrus is among the important production practices in citriculture (Anon., 1985). _Tylenchulus semipenetrans_ was discovered in 1912 by J. R. Hodges in citrus

plantings in Riverside, California (Thomas, 1913). This nematode has since been reported in all citrus producing regions of the world (Heald and O'Bannon, 1987; Van Gundy and Meagher, 1977). On virgin soil, the disease recognized as slow decline of citrus requires several years to debilitate trees and reduce fruit yield (Cohn et al., 1965; Reynolds and O'Bannon, 1963b). In contrast, replant disorders, particularly when the site is infested with high T. semipenetrans population densities, may kill young trees within the first year of replanting (Thorne, 1961). The mechanism whereby T. semipenetrans induces either disease is not known (Duncan and Cohn, 1990; Hamid et al., 198';).

Biology. The life cycle of T. semipenetrans consists of egg, juvenile (J1, J2, J3, J4), and adult stages (Cobb, 1914; Van Gundy, 1958), and is completed in 6-8 weeks, depending on the host and average soil temperature (Cohn, 1965; O'Bannon et al., 1966; Van Gundy, 1958). Each of the four juvenile stages is terminated by molting, with the first molt occurring within the egg (Gutierrez, 1947). Reproduction is parthenogenetic (Maggenti, 1981), and during its lifetime the mature female lays a total of ca. 500 eggs (Van Gundy, 1958) in a protective gelatinous matrix, which together with its contents are collectively termed an egg-mass (Maggenti, 1962).

All stages, except the J1 stage and adult males, parasitize root parts (Van Gundy, 1958). The J2, J3, and J4 stages feed on epidermal and hypodermal cells (Cohn, 1965;

Schneider and Baines, 1964; Van Gundy and Kirkpatrick, 1964). Juveniles and young females penetrate roots, with females eventually establishing feeding sites, consisting of 6-10 "nurse" cells around the nematode head (Van Gundy, 1958). The "nurse" cells are required for reproduction and die upon the female's death (Cohn, 1965). The "nurse" cells are similar in shape and size to the adjacent untransformed cortical parenchyma cells, but have different reactions to stains (Cohn, 1965; Kaplan, 1981; Van Gundy and Kirkpatrick, 1964). Heavily infected roots may accommodate ca. 100 females/cm of feeder root (Cohn, 1972). Root penetration may extend to the endodermis (Cohn, 1964; Van Gundy, 1958); however damage has to date been observed exclusively in the cortex. Infected roots are usually lesioned and appear darker than noninfected roots. Under high infection levels, the cortex along the affected region sloughs off, resulting in death of the affected rootlet (Cohn, 1965; Reynolds and O'Bannon, 1963).

Damage threshold. The damage threshold level of T. semipenetrans to citrus is not known, but estimates of population densities below which infected trees do not respond to nematicidal treatments in certain regions are available. The nematicidal response threshold densities for South Africa and Israel are ca. 4,000 juveniles/g fresh roots (Cohn, 1976), for California ca. 700 females/g fresh roots (Hamid et al., 1985), and for central ridge of Florida ca. 2,000 juveniles/100 cm^3 soil (Duncan and Cohn, 1990).

Damage symptoms. The initial clinical symptoms of T. semipenetrans damage are reduced terminal growth (Thomas, 1913). This is followed by leaf chlorosis, leaf abscission, die-back of young twigs, and smaller leaves and fruit. These symptoms are most noticeable in the uppermost portion of the trees. Slow decline symptoms vary with soil environment. A Californian citrus orchard with heavy infestations of T. semipenetrans, had no decline symptoms while trees in an adjacent orchard with comparable nematode densities had severe decline symptoms (Harding, 1954). Harding (1954) asserted that the soils in the two orchards were different without describing the nature of the differences. In the central ridge of Florida, with deep, well-drained sandy soils, T. semipenetrans population densities on mature trees may exceed 5,000 juveniles/g fresh roots, without any decline symptoms (O'Bannon, 1968). In contrast, in the poorly drained soils of the eastern coast of Florida, T. semipenetrans population densities below 1,000 juveniles/g fresh roots may induce severe decline symptoms.

Environmental factors. Tylenchulus semipenetrans population densities may have no specific period of active increase per annum (Cohn, 1966), or may have one (Bello et al., 1986; Prasad and Chawla, 1965), or two (Baghel and Bhatti, 1982; Duncan and Noling, 1988a; O'Bannon et al., 1972; Salem, 1980; Vilardebo, 1964). The Floridian T. semipenetrans female has the highest rate of development

(O'Bannon et al., 1972; Duncan and Cohn, 1990) in summer through autumn (July-Nov); whereas development decreases in winter (Dec-March). Although soil population densities increase in spring (April-May), development remains low (O'Bannon and Stokes, 1978). Causes of these periodicities in population densities have not been resolved.

Machmer (1958) in Texas recovered high T. semipenetrans densities from mature citrus irrigated with NaCl, Na_2SO_4, $CaCl_2$, $CaCl_2$/NaCl, and $CaCl_2$/Na_2SO_4 solutions, each with an electrical conductivity (ECiw) 6.5 dS/m over a 3-year period. In contrast, lower population densities were recovered from trees irrigated with surfacewater (Ec_{iw} = 2.5 dS/m). The highest T. semipenetrans population densities (4,0000-10,000 juveniles/g fresh roots) in the central Transvaal and the eastern Cape in South Africa; whereas the lowest densities (100- 500 juveniles/g fresh roots) were commonly recovered from the eastern Transvaal and the western Cape (Cohn, 1976). The high nematode densities in these major citrus-producing regions were associated with saline conditions; whereas low population densities were associated with nonsaline conditions (Cohn, 1976). Nematode surveys in Israel also showed that the highest population densities of T. semipenetrans occur in the more saline coastal or desert regions (Cohn et al., 1965; Heller et al., 1973). In fallow soil however, salinity reduced juvenile eclosion and infectivity of T. semipenetrans, but when salinity was removed, both activities were restored

(Kirkpatrick and Van Gundy, 1966). Viglierchio et al. (1969) showed that the osmotic potential threshold for reduction of _T. semipenetrans_ juvenile motility was -1.01 MPa; whereas at -4.05 MPa motility was completely inhibited.

Van Gundy and Martin (1961) found higher _T. semipenetrans_ population densities in alkaline than acid soils. The optimum soil reaction for _T. semipenetrans_ development is pH 6.0-7.5 (Van Gundy et al., 1964), but infection may occur under low soil reactions (Bello et al., 1986; Davide, 1971; Martin and Van Gundy, 1963; Reynolds et al., 1970). Optimum mean temperature (O'Bannon et al., 1966) for population development is 25 C (range 20-31 C). Juveniles are not active when mean soil temperature is below 16 C (Van Gundy, 1984). In contrast to _Meloidogyne_ spp., _T. semipenetrans_ population densities increase less rapidly in sandier soils, and more rapidly in soils with moderate percentages of clay and silt (Bello et al., 1986; Davide, 1971; Van Gundy et al., 1964). Excess soil moisture generally reduces _T. semipenetrans_ population densities, possibly through the reduction of soil O_2 (Norton, 1978). However, heavy rains interrupted by short drought spells increase population densities of _T. semipenetrans_, possibly by washing juveniles and eggs out of the gelatinous matrix (Ayoub, 1980). O'Bannon (1968) observed that in soils with 3-9% organic matter where this forms a thin protective layer around infected roots, _T. semipenetrans_ females accumulate in the greatest numbers. Tree age may also

regulate distribution and densities of _T_. _semipenetrans_ (Bello et al., 1986; Cohn et al., 1965; Sharma and Sharma, 1981). The population growths slowly in young trees until the canopies shade the soil, thus reducing wide fluctuations in average soil temperature (Reynolds and O'Bannon, 1963a). Similarly, old trees with advanced slow decline symptoms may harbor fewer nematode densities/unit soil than soil with healthy trees (Reynolds and O'Bannon, 1963b).

Dispersal. Adult _T_. _semipenetrans_ females are sedentary (Van Gundy, 1958); whereas the juvenile's active motility through the soil is negligible for dispersal. Tarjan (1971) demonstrated that horizontal movement of juveniles in Parkwood fine sand averaged 17.8 cm and in Lakeland fine sand 26.7 cm per year. Likewise, Baines (1974b) found that vertical movements of _T_. _semipenetrans_ juveniles in soils were limited. Thus, long distance dispersal is exclusively passive (Norton, 1978). Limited movement of _T_. _semipenetrans_ renders exclusion in noninfested sites the best management option for this parasite; whereas nematicides and resistant rootstocks are common management options for _T_. _semipenetrans_ in existing plantings and (or) in sites with citrus old soil (Duncan and Cohn, 1990).

Resistance. The availability of resistant germplasm to _T_. _semipenetrans_ was first reported in trifoliate orange, _Poncirus_ _trifoliata_ (L.) Raf. in Argentina (DuCharme, 1948). During the same period, Baines et al. (1948) demonstrated that

T. semipenetrans could not successfully complete its life cycle in 20 selections of *P. trifoliata* and Chinese box orange, *Severinia buxifolia* (Poir.) Ten. Subsequent studies (Baines et al., 1960; Cameron et al., 1954; Feder, 1968; Hutchison and O'Bannon, 1972; Hutchison et al., 1972; Newcomb, 1978) confirmed the presence of resistance to *T. semipenetrans* in certain genera and hybrids of subtribe Citrinae. The most resistant genus, *Severinia*, is not commercially used because sweet oranges grafted on *Severinia* spp. were found to be more sensitive to tristeza than on any other rootstock (Grant and Costa, 1949). Swingle citrumelo (*C. paradisi* x *P. trifoliata*), highly resistant to *T. semipenetrans* (Kaplan and O'Bannon, 1981), is currently the recommended rootstock for most Florida conditions (Castle et al., 1989). *Poncirus trifoliata*, although highly resistant to this nematode (Van Gundy and Kirkpatrick, 1964), the rootstock is not recommended in Florida (Castle et al., 1989) and Texas (Peynado and Young, 1969) because of its dwarfing effect on the scion and its unusual sensitivity to Cl toxicity (Cooper et al., 1951). In South Africa, *P. trifoliata* is used most commonly in old citrus soil (Von Broembsen, 1984).

Some of the mechanisms of resistance to *T. semipenetrans* in citrus rootstocks have been identified. Van Gundy and Kirkpatrick (1964) identified three mechanisms: (1) formation of hypersensitivity, (2) formation of wound periderm, and (3) production of toxins. Hypersensitivity reactions occur in

highly resistant rootstocks, and are associated with differential resistance, which is characterized by a large, monogenic effect (Fry, 1982). Poncirus trifoliata and its hybrid Swingle citrumelo (C. paradisi x P. trifoliata) possess the characteristics of differential resistance (Kaplan, 1981; Van Gundy and Kirkpatrick, 1964). On the other hand, nondifferential resistance, characterized by a smaller, polygenic effect (Fry, 1982), appears to exist in moderately T. semipenetrans resistant Carrizo and Troyer citranges (Kaplan, 1988).

Kaplan (1981) described five cellular resistant responses in six Citrinae genotypes and two T. semipenetrans biotypes, with wound periderm formation consistently following hypersensitive reactions. Kaplan (1981) proposed that the two responses were either genetically or functionally coupled. In fungal infection, cells adjacent to the infected ones play a major role in the host defense mechanism (Keen and Bruegger, 1977). These metabolically active cells play a role in the transport of biosynthesized intermediates and phytoalexins to the infected site. Kaplan (1981) proposed that the cells that form wound periderm in nematode-resistant rootstocks were functionally similar to those involved in resistance to fungal infection.

Plant resistance is predominantly an active process, and thus can be overcome (Kaplan and Davis, 1987). Fry (1982)

asserted that "breakdown of resistance" refers to a change in the pathogen population genes rather than a change in plant resistance genes. Although the breakdown of resistance in soybean cultivars by Heterodera glycines Ichinohe had been attributed to the exclusive change in nematode genes (Triantaphyllou, 1987), in other cases it appears that the plant itself may be induced to reverse its resistance to pathogens. For instance, high ambient temperatures (Dropkin, 1969) and foliar application of cytokinins (Dropkin et al., 1969) reversed resistance to Meloidogyne spp. in tomato plants. Also, Meloidogyne-Fusarium interactions reduced resistance to Fusarium spp. in tomato (Harrison and Young, 1941) and muskmelon (Bergeson, 1975) plants. The mechanisms involved in reversing resistance to pathogens in these studies have not been resolved. Because resistance is an active process (Kaplan and Davis, 1987), it is metabolically sustained. The energy demands for ion uptake and exclusion (Rains, 1968; Greenway and Munns, 1980), for cellular responses in plants under salinity stress (Poljakofi-Mayber, 1975) and possibly for nematode development, suggest that resistance to T. semipenetrans in Citrinae rootstocks can also be reversed when host plants are subjected to conditions where demands for metabolites are excessive.

Biotypes. The trifoliate oranges are not resistant to all T. semipenetrans populations in California (Baines et al., 1969). This has since been attributed to the presence of T.

semipenetrans biotypes. Baines et al. (1969) discovered four T. semipenetrans biotypes in California, and designated them as C1, C2, C3, and C4 biotypes. The existence of T. semipenetrans biotypes has since been confirmed in Florida (O'Bannon et al., 1977), Israel (Gottlieb et al., 1986), and Italy (Lamberti et al., 1976). In nematology the term biotype, as opposed to pathotype (race) in pathology, specifically refers to phenotypically similar nematode species, which reproduce parthenogenetically, but can be separated using differential host preferences (Triantaphyllou, 1987). The recognition of the presence of biotypes is important in the selection of resistant germplasm (Baines et al., 1969) and in enforcing quarantine regulations (Inserra et al., 1988).

Inserra et al. (1980) using differential host preferences separated four widely distributed T. semipenetrans biotypes. The biotype that hardly reproduced on P. trifoliata, but prolifically reproduced on citrus species, 'Carrizo' and 'Troyer' citranges, olive, grape and persimmon, was designated the 'citrus biotype'. Tylenchulus semipenetrans populations in Arizona, Florida, Texas, and the biotypes C1, C2 and C4 in California, were classified the 'citrus biotype'. The 'Poncirus biotype' (C3), indigenous to Japan, infected Poncirus and hybrids, citrus species, grape, and persimmon, but not olive (Inserra et al., 1980). The 'Poncirus biotype', in addition to the indigenous 'Mediterranean

biotype', also occurs in Israel (Gottlieb et al., 1986). The 'Mediterranean biotype', indigenous to citrus-producing regions with Mediterranean climate, is similar to the Indian and South African biotypes (Inserra et al., 1980). This biotype is closely related to the 'citrus biotype', except that it does not infect olive.

The Floridian 'grass biotype', reported on grass, Andropogon rhizomatus (Stokes, 1969), does not infect Citrinae. The 'grass biotype' has since been separated and described as two species, T. graminis and T. palustris (Inserra et al., 1988) based on morphological and differential host preferences. Description of the 'grass biotype' as two species increased Tylenchulus spp. to four: T. furcus, T. graminis, T. palustris, and T. semipenetrans (Inserra et al., 1988).

Interactions with ions. Van Gundy and Martin (1961) found that under high soil reaction and high soil K, sweet orange seedlings infected with T. semipenetrans accumulated more Na in leaves than the noninfected controls. Van Gundy and Martin (1961) also observed limited growth depression due to nematode infection in plants growing in soils with low K relative to those with high K levels. The suppression of shoot growth in soils with high K due to T. semipenetrans infection of roots was comparable to growth reduction due to Na toxicity in soils with high Na. Citrus foliar K deficits in South African orchards with high soil K, were related to T.

semipenetrans infection (Fouche et al., 1977; Milne and Willers, 1979). In both cases, reducing nematode levels with nematicides followed by fertilization, corrected the K deficiency, whereas fertilization without reducing high nematode densities did not ameliorate K deficiencies.

Tylenchulus semipenetrans-infected roots have lower concentrations of K, Cl, and Na than noninfected roots (Labanauskas et al., 1965). Van Gundy and Martin (1961) and Tarjan and O'Bannon (1984) proposed that the higher leaf Na and reduced leaf K in T. semipenetrans-infected citrus trees were due to chemical and (or) physical changes on root cell membranes by nematodes, concomitant with loss of ion selectivity.

Tylenchulus semipenetrans parasitism also decreased B, Cu, Mn, and Zn in citrus leaves (Elgindi et al., 1967; Embleton et al., 1962; Milne and De Villiers, 1978; Milne and Willers, 1979; Van Gundy and Martin, 1961). However, Labanauskas et. al. (1965) proposed that the magnitude of nutrient ion imbalances in T. semipenetrans-infected citrus plants were too small to account for any stunted growth.

Hamid et al. (1985) proposed that above 700 females/g fresh roots T. semipenetrans infection depleted shoot carbohydrates. Others (Crider, 1927; Krishnamurthi et al., 1960) observed that infected roots were characterized by repeated root regeneration, which Hamid et al. (1985) used as evidence to support their tentative hypothesis which proposed

that the clinical symptoms of slow decline were due to depletion of carbohydrates required to support shoot growth. The typical clinical symptoms associated with an extreme case of shoot carbohydrate depletion are those in the tree collapse of 'Murcott' Tangerines (Smith, 1976). The early clinical symptoms are wilting, chlorosis, defoliation, and fruit shrivelling. The late symptoms include rapid abscision of leaves in all growth stages, fruit drop, and culminating with die-back of branches. In the advances stages of 'collapse' the trees have a withered appearance. The symptoms associated with excess depletion of nonstructural carbohydrates in shoots are clearly different from those induced by T. semipenetrans parasitism (Cobb, 1914; O'Bannon and Esser, 1985; Thomas, 1913). As in T. semipenetrans infection, collapsed trees have deficiencies in K, Mn, and Zn; however, fertilization cannot curtail tree 'collapse' (Smith, 1976). Another disease of citrus, the 'collapse of lemons on sour orange rootstocks' (Rodney et al., 1956), was also ascribed to the reduction of nonstructural carbohydrates in roots due to phloem necrosis above the bud union. In the advanced stages of this collapse, affected trees have high Na in root and leaf tissues, and lower K and P in leaves.

The clinical symptoms of T. semipenetrans parasitism and salinity stress each include stunted growth, leaf chlorosis, smaller leaf and fruit size, die-back of young twigs, and defoliation (Anderson, 1985; Cohn, 1972; Cooper, 196; Maas,

1993; O'Bannon and Esser, 1985; Tarjan and O'Bannon, 1984; Thorne, 1961). These symptoms are similar to those induced by several nutrient element deficiencies (Levitt, 1980). The similarity among the symptoms of T. semipenetrans, salinity, and nutrient deficiency, suggest a potential common link among these stresses. For example, leaf chlorosis is typical of Fe deficiency and Cl toxicity (Cooper and Peynado, 1959; Embleton et al., 1962; Wutscher, 1979); die-back of young twigs is typical of Cu and Mn deficiencies (Anderson, 1985); smaller leaves and fruit are common under K deficits (Chapman et al., 1947 ; Jones and Cree, 1953); whereas defoliation is typical of Cl and (or) Na toxicities (Cooper, 1961).

Salinity in citrus production

Three salt groups associated with agricultural salinity are chlorides, sulfates, and carbonates (Levitt, 1980). The chloride salts have higher solubilities in water: $CaCl_2$ 25,470 mols/m^3, $MgCl_2$ 14,955 mols/m^3, and NaCl 6,108 mols/m^3, than the sulfates, $MgSO_4$ 5,760 mols/m^3 and Na_2SO_4 683 mols/m^3, and the carbonate, $NaCO_3$ 1, 642 mols/m^3 (Doneen, 1975).

Because the Cl salts are the most soluble in water, the Cl ion is the most commonly found anion in irrigation water (Bohn et al., 1985; Waisel, 1972); whereas Na is the dominant cation because it is the lowest on the lyotropic series (Bohn et al., 1985; Sposito, 1989). Chloride and Na are thus the dominant saline ions in soil solution (Bohn et al., 1985).

Salinity, described as a condition where excessive salt accumulation in the root zone impede plant growth (Bohn et al., 1985), can be quantified in units of electrical conductivity of soil extract (Ec_e), sodium adsorption ratio (SAR), and soil reaction (Bohn et al., 1985). Nonsalinity is the soil condition where $Ec_e < 4$ dS/m, SAR < 15, and pH < 8. In contrast, salinity is a condition where $Ec_e > 4$ dS/m, SAR > 15, and pH < 8. High Na or sodicity (Sposito, 1989) is defined by $Ee_e > 4$ dS/m, SAR > 15, and pH > 8 (Bohn et al., 1985; Sposito, 1989).

Salinity-inducing salts enter into soil solution through fertilizers, debris decay, weathering of soil parent materials, irrigation with saline water, or rain in regions with polluted atmospheres (Bohn et al., 1985; Epstein et al., 1980; Levitt, 1980; Sposito, 1989). The major contaminants of irrigation water with saline ions include erosion of parent materials and fertilizers, excess leaching, encroachment of sea water into groundwater, and domestic and industrial wastewater (Bohn et al., 1985).

Yield reduction. Salinity studies in citrus have mainly comprised NaCl salt, presumably because the two ions are the most common in soil solution (Bohn et al., 1985; Sposito, 1989). Bernstein (1969a) estimated that 10-15 mols $NaCl/m^3$ H_2O salinity can reduce mean citrus yield by 10%; whereas Chapman et al. (1969) estimated 10-20% yield reduction at -7 mols $NaCl/m^3$ H_2O. Heller et al. (1973) in a 5-year-study using 9

mols NaCl/m^3 H_2O on a 10-year-old Shamouti grafted on sour orange ascribed a 20% reduction in yield to salinity. The average salinity threshold damage for citrus is low, 1.4 dS/m, with 13% reduction in yield for every unit increase above this threshold (Maas, 1993). Thus, yield losses due to salinity are comparable to those reported for *T. semipenetrans* parasitism, which averages 14% (Anon., 1985). Citrus trees under salinity also produce fruit with low quality juice (Levy and Shalhevet, 1990; Nieves et al, 1991b).

Salt source. Strogonov (1962) and Poljakoff-Mayber (1975) argued that SO_4 salinity was the most typical of natural conditions and that it was the most damaging to plants. Peynado and Young (1963) showed that the severity of salt-induced chlorosis in Cleopatra mandarin and sour orange seedlings was in the order $CaCl_2$ > Na_2SO_4 > NaCl in both sand and loam soils. El-Azab et al. (1973) confirmed that chlorosis and marginal leaf burn on Cleopatra mandarin and sour orange seedlings were more pronounced where seedlings were treated with SO_4 salts than with Cl salts. In contrast, the severity of bronzing was in the order of $CaCl_2$ NaCl > Na_2SO_4 (Peynado and Young, 1963). Peynado and Young (1963) found that bronzing under $CaCl_2$ or NaCl salinity was followed by leaf abscission and twig die-back in loam soil grown trees; whereas leaf abscission alone occurred in sand. Sodium sulfate salinity did not induce leaf abscission or die-back of twigs in both soil types. Overall, NaCl and Na_2SO_4 each

reduced citrus growth more than $CaCl_2$ (Peynado and Young, 1963). However, Hewitt and Furr (1965) found that Na_2SO_4 salinity was less damaging to citrus than NaCl.

Salt source also influences the amount and the type of ion accumulation in citrus leaves. Relative to NaCl salinity, more Cl and less Na accumulated in leaves of trees under $CaCl_2$ and Na_2SO_4, respectively (Brown et al., 1953; Peynado and Young, 1963). Hayward and Wadleigh (1949) demonstrated that SO_4 inhibited Ca uptake, whereas it promoted Na uptake. However, Zusman (1956) found no evidence of Ca deficiency in citrus seedlings with visual SO_4 toxicity symptoms. Brown et al. (1953) demonstrated that by enhancing Na uptake, SO_4 may induce Na toxicity in Na sensitive species. However, when SO_4 was applied as Na_2SO_4, SO_4 accumulation in citrus paralleled Na accumulation, with no SO_4 toxicity even under high levels of Na_2SO_4 (Cooper, 1961). Boron contaminated NaCl solutions on sweet oranges resulted in Cl but no B accumulation, and vice versa; whereas _S. buxifolia_ excluded both Cl and B ions (Cooper, 1961).

Damage threshold. Bingham et al. (1973) proposed that the damage threshold Ec_e for mature citrus trees was 3.0 dS/m; whereas Mass and Hoffman (1977) suggested 1.8 dS/m for grapefruit and 1.7 dS/m for orange trees. Recently, (Maas, 1993) proposed that the damage threshold for citrus is 1.4 dS/m, with 13% decrease in yield for every additional 1 dS/m above 1.4 dS/m.

Irrigation water recommended for citrus production under most conditions (Marsh, 1973) has Ec_{iw} below 0.75 dS/m [Total soluble salts (T.S.S.) = 480 ppm]. Water with Ec_{iw} > 2 dS/m (T.S.S. = 1,280 ppm) is unsuitable for citrus production under all conditions; whereas water with Ec_{iw} 0.75-2.00 dS/m is marginal (Marsh, 1973).

Citrus yield is reduced if the exchangeable sodium percentage (ESP) of the soil is above 6% (Martin et al., 1961; Pearson and Huberty, 1959). Exchangeable Na percentage above 15% causes flocculation, which is a process where clay particles absorb Na, and when the soil dries it expands resulting in the deterioration of soil structure (Bohn et al., 1985; Sposito, 1989). Sodium adsorption ratio (SAR), which is an estimate of the ESP attained in soil at equilibrium with irrigation water (Bohn et al., 1985; Sposito, 1989), is unlikely to create an excess of exchangeable Na in the soil when it is below 4 (Marsh, 1973). In contrast, water with SAR above 8 consistently produced an ESP injurious to both citrus and soil structure; whereas water with SAR 4-8 was marginal for both citrus yield and soil structure (Marsh, 1973).

Harding and Chapman (1951) proposed that Cl in citrus leaves was physiologically toxic at 0.25% Cl dry leaf tissue basis. Hayward and Bernstein (1958) noted that ca. 1.00% Cl in leaves was the Cl-toxicity danger zone, whereas under South African conditions Robinson (1981) suggested 0.70% Cl. The minimum foliar Cl associated with visible leaf-burn symptoms in citrus

is within 1.35%-2.77% Cl; whereas lower levels induce bronzing (Cooper et al., 1951; Cooper et al., 1952). These limits may vary with the rootstock vigor. Vigorous rootstocks tend to induce active scion growth, which continues to dilute Cl in leaves (Peynado and Young, 1962). Leaves of sweet orange grafted on C. macrophylla rootstock, for instance, may contain up to 2.54% Cl (dry weight) without visible Cl toxicity (Peynado and Young, 1962).

Chloride-toxicity in citrus leaves results in both chlorosis and bronzing (Peynado and Young, 1963), but without well-defined necrotic lesions (Bernstein, 1969). Chlorosis occurs first, and then the residual yellow color (carotenoids) becoming modified by a bronzing of the chlorotic area (Bernstein, 1969). Symptoms are usually more severe on sun exposed leaves than on shade leaves.

Sodium-toxicity in citrus causes well-defined necroses in isolated areas along the leaf margins and tips (Bernstein, 1965). In contrast to Cl-toxicity which results in both chlorosis and bronzing, Na toxicity induces chlorosis only (Peynado and Young, 1963). Physiological damage threshold for Na in citrus leaves is ca. 0.10% Na; whereas visual symptoms usually occur at ca. 0.25% Na (Cooper et al., 1952; Robinson, 1981). Recent studies (Syvertsen et al., 1988) demonstrated that excess Na in leaves is physiologically more toxic to citrus than excess Cl. Because Cl and (or) Na toxicity inevitably result in leaf chlorosis and (or) abscission, the

two ions reduce the effective lifespan of citrus leaves. Thus, whereas studies on the physiological effects of salinity concentrate on surviving leaves, salinity defoliated leaves should not be neglected.

The SO_4 toxicity symptoms consist of yellowing of the leaf margins (Zusman, 1956). Prior to necrosis, chlorosis spreads interveinally toward the midrib.

The effect of $NaCO_3$ salinity on plants is mainly through increasing the Na hazard in soils (Bohn et al., 1985; Sposito, 1989). The bicarbonate ion in soil solution reacts with Ca to form a nonexchangeable $CaCO_3$ precipitate (Bohn et al., 1985). Precipitation of $CaCO_3$ reduces the concentration of Ca in soil solution, thus increasing SAR, which implies an increase in exchangeable Na of soil solution (Bohn et al., 1985), resulting in the reviewed Na hazards. The increase in soil reaction under carbonate salinity may also induce nutrient deficiencies in plants (Bohn et al., 1985).

Salt tolerance in citrus. Although the subtribe Citrinae is relatively sensitive to salinity (Shalhevet and Levy, 1990), certain genera have limited abilities to tolerate salinity (Maas, 1993). Salt tolerance in citriculture is defined as the ability of roots to exclude excess Cl and (or) Na ions from shoots (Castle et al., 1989; Cooper, 1961).

The first report on salt tolerance in *Citrus* spp. was in Marsh grapefruit grafted on *S. buxifolia* in Riverside, California (Webber, 1948). Because sweet oranges on *S.*

buxifolia were more susceptible to tristeza virus than on other rootstocks (Grant and Costa, 1949), attempts to further evaluate S. buxifolia for commercial were not pursued.

Cooper and co-workers in Texas pioneered the screening of the subtribe citrinae for salt tolerant germplasm using S. buxifolia as a standard. Salt tolerance to Cl ions in Cleopatra mandarin and Rangpur lime was comparable to that of S. buxifolia; whereas the citrange and trifoliate oranges were the least tolerant to Cl. Cleopatra mandarin was, however, more susceptible to Na than the trifoliate oranges. Cooper (1961) also demonstrated that while Macrophylla was highly tolerant to Na, it was nonetheless highly susceptible to the Cl ions. Cooper et al. (1951) should, accordingly, be credited with the observation that no single Citrinae rootstock is capable of excluding both Cl and Na.

Broadly, leaf Cl concentrations increased in the order mandarins < sweet oranges < trifoliates; whereas Na increased in the order sweet oranges < trifoliates < mandarins (Cooper, 1961). Short-term salinity in soil solution, regardless of the degree of salt tolerance in the rootstock, results in higher Na in feeder roots than leaves; whereas the opposite is true for Cl (Cooper et al, 1952). In Florida (Syvertsen, 1990), Texas (Peynado and Young, 1969), Israel (Beloirai et al., 1988), Spain (Carpena et al., 1969; Nieves et al., 1991b), and probably most other citrus-producing areas, salinity is a periodic problem, with high salt levels

accumulating in the root zone during irrigation seasons, and being leached out in rainy seasons.

The scion appears to have little, if any, role in exclusion of excess Cl and (or) Na from leaves (Behboudian et al., 1986; Cooper et al., 1952). The rootstock is the major regulator in exclusion of excess Cl and Na from shoots (Behboudian et al., 1986; Storey and Walker, 1987; Walker et al., 1983). However, the exact location or mechanism involved in the exclusion of either ion has not been resolved. In other crops exclusion of Cl or Na may be in the xylem of the roots into the corticular vacuoles (Greenway et al., 1981), and Na may also be removed from the xylem of the stem into the phloem and translocated to roots (Kramer et al., 1977; Lauchli et al., 1974; Lauchli and Wieneke, 1978), or under low concentrations of Na in shoots, it may be exported from shoots to roots (Greenway and Munns, 1980).

Essential roles of Cl and Na. An essential plant nutrient element is the ion without which a plant cannot successfully complete its normal life cycle (Epstein, 1972). Small quantities of Cl (2 ppm) in the soil are required by vascular plants as an essential plant nutrient (James et al., 1970). Chloride plays a role in the evolution of O_2 in photosystem II during CO_2 assimilation (Bove et al., 1963). Because the Cl levels in the atmosphere, eventually washed into the soil by rainfall, are high enough to meet the 4-10 kg/ha/year required by higher plants (Reisenauer et al.,

1973), it is rare for Cl deficit to occur in plants. Under controlled conditions, the clinical symptoms of Cl deficiency are chlorosis in young leaves and an overall wilting of the plant (Broyer et al , 1954; Johnson et al., 1957; Ulrich and Ohki, 1956). The critical Cl deficiency range in plants is 0.007-0.01 Cl (70-100 ppm Cl) dry tissue basis.

Sodium is an essential nutrient for some C4 plant species (Brownell and Crossland, 1972). Sodium increases the activity of phosphoenolpyruvate (PEP) carboxylase (Shomer-Ilan and Waisel, 1973), which is the primary carboxylating enzyme in C4 photosynthesis.

Together with K (Salisbury and Ross, 1985), the major nonessential role of both Na and Cl is in regulating osmotic potential of cells (Mengel and Kirkby, 1978; Waisel, 1972), and thus, are collectively called osmotically active ions. The osmotically active cells may affect plant growth through their influence on water potential of cells. For instance, decreasing water potential reduces plant growth. Water potential threshold levels where plant growth ceases have been characterized for certain plant species (Boyer, 1970; Gandar and Tanner, 1976; Hsaio, 1973; Kanemasu and Tanner, 1969). Generally, growth stops at -0.65 MPa cellular water potential (Hsaio et al., 1973).

<u>Physiological effects</u>. Removal of Ca from root tissues using ethylenediamine tetraacetic acid (EDTA) reduced the ability of root cells to absorb and retain ions (Hanson,

1960). Epstein (1961) showed that Ca was indispensable for normal cation absorption by roots. Also, the selectivity of K over Na is Ca-dependent (Epstein, 1961). Currently, it is recognized that a solution containing Ca is a required physiological ion meliu for plant tissues (Maas, 1993). This requirement is not exotic because Ca has the highest concentrations of all ions in most agricultural soils (Bohn et al., 1985; Sposito, 1989). Others (Elzamand and Hodges, 1967; Falade, 1973; Gauch, 1972; Minchin and Baker, 1973) showed that high concentrations of Ca prevented unusually high rates of monovalent cation absorption by roots. For instance, increased Ca levels in irrigation solutions were shown to reduce leaf K in grapefruit grafted on Cleopatra mandarin and sour orange (Gordon et al., 1954). Notwithstanding the side effects, appreciable concentrations of Ca in soil solution is the normal physiological condition for roots.

Interactions with nutrient ions. Soil salinity can upset balance of nutrient ions in plant tissues (Levitt, 1980). The first report on salinity-nutrient interactions in citrus was the reduction of foliar K by NaCl salinity (Cooper and Gorton, 1952). Gorton and Cooper (1954) demonstrated that $CaCl_2$ salinity increased leaf Ca; whereas it reduced foliar K in grapefruit on both Cleopatra mandarin and sour orange rootstocks. Many workers (Alva and Syvertsen, 1991; Behboudin et al., 1986; Nieves et al., 1990, 1991a; Syvertsen et al., 1988; Zekri, 1988) have since confirmed that salinity stress

consistently reduces foliar K. Also, relative to unsalinized controls, salinity reduced K in roots (Behboudin et al., 1986). Alva and Syvertsen (1990) found that leaf P on mature trees was higher under NaCl salinity relative to unsalinized controls. The effect of salinity on other nutrient elements is variable.

Ion movements in roots. Organization of cells in roots is closely related to ion absorption and transport to the root xylem vessels. Various cell types are integrated in such a way that ion transport consist of an overall capability of the entire root system.

Ion uptake by roots is closely related to the properties of the root surface and the cortical cells in direct contact with soil solution. The root surface varies greatly with the developmental stages along the distal region of the root tip. Root cap cells decompose and budd off to provide a slime cylinder in which the root proliferates with minimal damage to the delicate zone of cell division. The slime also provides a mucigel in which the root can establish intimate contact with soil particles. Mucigel may also enhance the adsorption exchange capacity of soil particles thus increasing ion availability in the soil solution (Marschner, 1985). Microorganisms growing in the mucigel also play a role in soil-root interactions (Nissen, 1973).

The root surface from the zone of cell division to the zone of cell differentiation is enclosed by the epidermal

layer, which consists of single closely packed living cells (Campbell, 1990). The epidermis is the first semipermeable barrier to ion diffusion (Bange, 1973). The epidermal and cortical cells of roots are interconnected by plasmodesmata to form the cortical symplasm (Robards, 1971). In the zone of cell differentiation, the epidermal cells grow out to form root hairs, whose cell walls largely consist of pectic acid (Campbell, 1990), through which another close adsorption exchange with soil particles is possible. The root hairs increase the absorption surface of roots. In the basal parts of the zone of cell differentiation, the root surface is suberized and cutinized, thus creating an impermeable barrier to ion and water movement (Leggett and Gilbert, 1969).

Plant species with limited root hairs such as citrus, have developed a mutual relationship with vesicular-arbuscular mycorrhizae (Harley and Smith, 1983; Maronek, 1981). The mycorrhizal hyphae spread among and into the cortical cells right up to the endodermis. The hyphae have arbuscular tufts of haustoria and vesicular storage organs in the root cells (Harley and Smith, 1983; Maronek, 1981). The hyphae also extend a few cm from the root surface into the soil, thus increasing the surface area in contact with the soil and also act as a pathway of nutrient and water from the soil to the root.

Radially, roots contain two morphologically distinct zones, the cortex and the stele. The cortex is exteriorly

bounded by the epidermal layer and interiorly by a single celled, endodermal layer (Campbell, 1990). The endodermal apoplastic pathway is completely sealed by the suberized Casparian strip (Campbell, 1990), thus forming an apoplastic barrier between the cortex and the stele. Both the outer and inner tangential walls of the root endodermis are penetrated by plasmodesmata so that the cortical and stelar symplasm are continuous through the endodermis (Helder and Boerma, 1969).

In contrast to the cortex which consists of parenchyma cells only, the stele has the pericycle cells, xylem and phloem parenchyma, xylem elements, phloem elements, and the central core of pith (Campbell, 1990). The parenchyma cells of the stele are well vacuolated and contain similar concentrations of K as cortical cells (Lauchli et al., 1971). The cytoplasm in xylem parenchyma cells contains the normal complement of mitochondria, with well developed endoplasmic reticulum, particularly adjacent to pits in the secondary wall (Lauchli et al., 1974). The xylem parenchyma may also have infoldings which are associated with transfer cells (Pate and Gunning, 1972). Both development of endoplasmic reticulum and cell wall-infoldings are involved in the secretion of ions from the stele to the xylem (Lauchli et al., 1974). The xylem parenchyma cells are also interconnected by plasmodesmata (Campbell, 1990). The epidermal, cortical, endodermal, and stelar parenchymal cells are therefore interconnected, to form a continuous symplasm from the roots through the stem to the

leaves. Subsequently, once an ion is absorbed by the epidermal or cortical cell from soil solution, it may be transported symplasmically to the leaves without entering the transpiration stream in the dead xylem vessels (Lauchli et al., 1974). Microscopic studies using precipitation techniques demonstrated that symplasmic transport is the major route of ion transport from the soil solution into the xylem vessels for most of the ions (Anderson, 1976). At low concentrations Cl transport in the cortex is exclusively symplasmic, while high concentrations use both pathways (Stelzer et al., 1975). On the contrary, Ca transport at any substrate concentration is exclusively apoplasmic.

Calcium at mM concentrations is cytotoxic because it precipitates P (Weber, 1976). Most cytoplasmic Ca is either bound or sequestered in the endoplasmic reticulum (Marme, 1983). The cytoplasm also actively pumps Ca into the apoplasm (Macklon and Sim, 1981), lowering symplasmic Ca to an average concentration of 0.1 mM, where reaction with P is negligible (Kretsinger, 1977). Calcium transport in the root is confined to the root tips (Harrison-Murray and Clarkson, 1973; Robards et al., 1973). Robards et al. (1973) demonstrated that the Casparian strip in the primary endodermis of the root tips also has an impenetrable barrier to apoplasmic Ca transport. Robards et al. (1973) demonstrated that the only way Ca ions can cross the endodermis is by diffusion through the tangential canals of the plasmodesmata connecting the cortex

and endodermis, after which it is released by the inner tangential canals into the apoplast of the stele. Because symplastic transport of ions is faster than apoplastic diffusion of ions, Robards et al. (1973) thus clarified the relative immobility of Ca in roots.

Calcium in irrigation water can mitigate the deleterious effects of Na on soil structure (Bohn et al., 1985) and overall ionic toxicity in plants (Epstein, 1961). Calcium amendments were demonstrated to improve citrus growth and prevented deterioration of soil structure in noncarbonated saline conditions (Cooper and Peynado, 1955). Calcium nitrate and $CaSO_4$ are the commonly used Ca amendments (Bohn et al., 1985). Cooper (1961) added NaCl and $CaCl_2$ in a 1:1 (w/w) ratio in irrigation solutions; whereas others (Alva and Syvertsen, 1991) used 3 parts NaCl and 1 part $CaCl_2$ (w/w) in NaCl studies.

<u>Vascular bundles</u>. The major cations in the transpiration stream, in decreasing order, are K, Ca, Mg, and Na; whereas the anions are P, Cl, S, and N (Jacoby, 1965; Wallace and Pate, 1967; Jones and Rowe, 1968), and some traces of B, Cu, Zn, Mn, and Fe (Husa and McIlrath, 1965). The translocation stream, on the other hand, has high concentrations of K, moderate concentrations of other ions, and traces of Ca, N, S, and B (Kimmel, 1962; MacRobbie, 1971). The concentration status of an ion in both the xylem and phloem is a measure of its relative mobility in plants (Pate, 1975).

<u>Leaf age</u>. Smith (1966) documented the effects of leaf age on the concentrations of Ca, Mg, N, P, and K in citrus. Briefly, Mg and Ca in citrus leaves are relatively low during leaf emergence, but increase with leaf age. Calcium continues to increase over an 11-month period; whereas Mg reaches the maximum level in 5-6 months of leaf development, and then declined so that 11 months after emergence it is low. In contrast, at leaf emergence, N, P, and K are high, but rapidly decrease 3 months after emergence. Soon after emergence, N and P increased and then stabilize until after 11 months where they decrease rapidly. Potassium follows the same trends as N and P, but declines rapidly 7-9 months after emergence, and then stabilizes in a deficient range. In contrast, young citrus leaves have lower Cl than old leaves (Syvertsen et al., 1988).

During leaf senescence the concentrations of P, N, K, Cl, and Mg in leaves of most plant species decrease noticeably; whereas the decrease of Ca, Mn, Zn, Fe, and B is negligible (Hes, 1958; Humphries, 1958a; Oland, 1963; Hart and Kortschak, 1965; McIlrath, 1965). Because of relocation of ions, the translocation stream has high levels of P, N, K, Cl, and Mg with the onset of leaf senescence in most plant species (Peel and Weatherley, 1959; Zimmermann, 1969). Apparently, in citrus foliar Cl and Na do not relocate, and leaf abscission appears the sole mechanism by which citrus decreases toxic levels of Cl and Na in shoots.

Root age. The most effective tissues through which ions in roots are transported to the stele were previously thought to occupy 1 cm of the apical meristems of root tips. This inference was made because that region accumulates ions rapidly (Steward and Sutcliffe, 1959; Bowen and Rovira, 1967; Rovira and Bowen, 1968), has high metabolic rates (Steward et al., 1942), and the fact that above this region the Casparian strip in the endodermis creates a barrier to apoplastic movement to the stele (Steward et al., 1942).

However, root age is known to affect the transport of Ca to the stele (Clarkson et al., 1968; Harrison-Murray and Clarkson, 1973; Russell and Sanderson, 1967). This is probably because of its apoplastic transport (Robards et al., 1973); suggesting that those ions that readily use the symplastic pathway, their rate of entry into the stele is not limited by root age (Clarkson et al , 1968; Harrison-Murray and Clarkson, 1973; Russell and Sanderson, 1967).

Mechanical root pruning

Mechanical root pruning is primarily a nursery cultural practice (Davidson and Mecklenburg, 1981; Eis, 1968; Harris et al., 1971). Inadvertent root pruning in citrus orchards occurs during mechanical weed cultivation. In nursery production, pruning generally produces a hardy plant with a high root:shoot ratio and a dense compact fibrous root system which can be transplanted easily. Such plants have high survival rates after transplanting (Mullin, 1966; Rohring,

1977; Sutton, 1967; Eis, 1968; Rook, 1971; Van Dorsser and Rook, 1972; Sweet and Rook, 1973; Tanaka et al. 1976).

Wilcox (1955) studied the histological responses to root pruning. Basipetally, from the pruned area, there are five distinct regions: (1) an outer zone of desiccated cells, (2) a zone infiltrated with wound substances showing disorganization and necrosis, (3) a zone of wound cork in the outer callus, and (4) a zone of meristematic callus, (5) a transition zone to normal tissue.

Generally, after roots have been pruned the remaining roots regenerate a bigger and denser root system than the unpruned roots (Wilcox, 1955). This is achieved by stimulating lateral root induction (Wilcox, 1955), which never originated beyond 5 mm from the excised area (Carlson, 1974; Wilcox, 1955). The capacity to induce lateral roots may be affected by the thickness of the pruned root. Root regeneration was enhanced on thinner apple roots (Gorbatyuk, 1975); whereas thicker grape roots regenerated better Oniani, 1973). New lateral roots appeared 3 days after pruning in pea (Torrey, 1950), red oak 4-5 days (Carlson and Larson, 1977), and in European birch 14 days (Kelly and Mecklenburg, 1980). Whereas the capacity to regenerate new laterals depends on plant species and root diameter, photoperiod also plays a major role.

As the day length decreased, the desirable effects of pruning on roots were nullified (Mullin, 1966; Van Dorsser and

Rook, 1972). Since photosynthesis rates are lower during short days, only a small amount of assimilates were translocated to roots, resulting in lower root:shoot ratios. Thus, plants should be pruned as the day length increases, especially when shoots are growing vigorously (Mullin, 1966). In fact, high survival rates after transplanting into the field also occur only when root pruning was initiated in spring; whereas fall pruning had the opposite effects (Mullin, 1966; Van Dorsser and Rook, 1972).

The immediate effect of root pruning is the reduction of the root:shoot ratio. Root:shoot ratio is a functional equilibrium between roots and shoots (Brauwer and DeWit, 1969). Under a specific set of environmental conditions, each plant species has a characteristic root:shoot ratio. Under stable conditions, this ratio remains constant, but progressively decreases with plant age and size (Kramer and Kozlowski, 1979). Soon after pruning, the plant suppresses shoot growth in favor of root growth (Alexander and Maggs, 1971; Haries et al., 1971; Richards and Rowe, 1977). Peach seedlings redistributed growth by increasing root weight by 20% while reducing shoot weight by 23% (Richards and Rowe, 1977). Similar redistributions of growth were observed in barley (Humphries, 1958a), apple (Taylor and Ferree, 1981), sweet orange (Alexander and Maggs, 1971), and tomato (Cooper, 1971) seedlings. Growth allocation to roots eventually

results in the reestablishment of the prepruned root:shoot ratios, concomitant with normal physiological functions.

The duration required to restore the root:shoot ratio depends on species and environmental conditions. For instance, for peach seedlings this duration is ca. 25 days (Richards and Rowe, 1977), carrot ca. 56 days (Benjamin and Wren, 1978), and pine seedlings ca. 80 days (Rook, 1971).

The mechanism whereby root-pruned plants suppress shoot growth in favor of root growth is not clear. However, Randolph and Wiest (1981) proposed that shoot growth may be suppressed by root pruning due to: (1) imbalances in hormones, (2) reduced CO_2 assimilation, (3) reduced transpiration, and (4) reduced nutrient ions via less uptake surface.

Hormones. Auxin activity in red oak roots quickly increased in the first 24 hours following pruning and then decreased to prepruning levels in less than 48 hours (Carlson and Larson, 1977). Dipping the remaining roots of root-pruned red oak seedlings in an auxin solution increased growth of lateral roots 24-fold (Carlson, 1974). Thus, the short-lived peak in auxin production (Carlson and Larson, 1977) confirmed the role of auxin as a trigger in inducing lateral root primordia (Torrey, 1950).

The root system is the major synthetic center of cytokinins (Skene, 1975; Wightman and Thimann, 1980; Wightman et al., 1980) and gibberellins (Butcher, 1963). These hormones are mainly produced in the root apices (Jones and

Phillips, 1966; Skene, 1975; Van Staden and Davey, 1979). However, roots are not the only centers of cytokinin production. For instance, Carlson and Larson (1977) observed high cytokinin concentrations in red oak seedlings with all root apices excised. It is probable that hormone precursors are produced in leaves and (or) buds, and then translocated to roots where they are converted into hormonal forms and then transported to shoots (Crozier and Reid, 1971; Kamienska and Reid, 1978). Notwithstanding these findings, root apices are the major centers for cytokinin synthesis. Higher quantities of cytokinin were extracted from 0-1 mm than 1-5 mm of the root apex (Short and Torrey, 1972; Weiss and Vaadia, 1965). Thus, a cytokinin deficiency may result when the root system is mechanically or parasitically reduced.

Applying cytokinin exogenously to leaves decreased root:shoot ratios; whereas application to roots increased the ratios (McDavid et al., 1973; Richards, 1980). Richards (1980) proposed that one of the roles of cytokinins was to draw photosynthates to the recipient of cytokinin. Thus, the proportion of photosynthates retained by shoots may depend on the amount of cytokinin supplied from root apices to the entire shoots (Richards, 1980; Richards and Rowe, 1977).

According to this hypothesis, a reduction in the cytokinin supply to shoots reduces the sink capacity of shoots for photosynthates; whereas it increases the sink capacity of roots. This mechanism may relate to the observed reduced

shoot weight and the increased root weight under root pruning.

Carbon dioxide assimilation. Carbon dioxide assimilation of pine seedlings decreased during the first 2 weeks after pruning, but progressively recovered until there were no differences between the pruned and unpruned treatments 4 weeks after pruning (Abod et al., 1979). In root-pruned gamagrass, CO_2 assimilation decreased during the first week following pruning, and slowly recovered thereafter (Detling et al., 1980). In root-pruned pea a consistent decline in CO_2 assimilation occurred until 16 days after pruning where it was 33-50% below that of unpruned controls, and then slowly approaches mean assimilation for control plants (McDavid et al., 1973). The reduction of CO_2 assimilation suggests that stomates close after root pruning. Because stomatal closure affects transpiration more than it affects CO_2 assimilation (Levitt, 1980), root pruning would thus reduce transpiration even more than assimilation.

Transpiration. Kramer and Kozlowski (1979) noted that when absorption lags behind transpiration, internal water deficits developed, resulting in the closure of stomates, and thus transpiration reduction. Stansell et al. (1974) found that when the availability of water in cotton was reduced by root pruning, transpiration of pruned plants remained below that of unpruned controls until the prepruning root:shoot ratio was reestablished. Kramer and Kozlowski (1979) asserted that plant growth is closely related to the availability of

water because a minimum water level is required for cell expansion. Randolph and Wiest (1981) showed that root pruning induced the development of internal water deficits in plants, which was quantitatively related to the reduced shoot growth.

Nutrient ions. The efficiency of roots in ion acquisition depends on the amount of root surface in contact with the soil and on the permeability of the root surface (Kramer and Kozlowski, 1979). All parts of the root system absorb most ions; whereas the rate is greatest for Ca in apical regions (Atkinson, 1980). Increased lateral roots following pruning may provide more apices, thus increasing the absorptive surface. The influence of pruning on root permeability has not been documented.

Studies on the effects of pruning on nutrient ions are few and the results contradictory. Root pruning had no influence on N content in barley 30 days after pruning (Humphries, 1958a) and pine seedlings 90 days after pruning (Stephens, 1964). The concentrations of N, P, and K, 18 days after under-cutting oak seedlings were lower than in the controls (Rohrig, 1977). Richards and Rowe (1977) found that root-pruned peach seedlings had higher N, P, K, and Ca in leaves 25 days after pruning. Faust (1980) found lower Ca in leaves 40 days after root pruning. Continuous excision of young roots resulted in reduced K uptake, possibly due to increased efflux as demonstrated by high levels of K in the growth medium on two occasions (Rees and Comerford, 1990).

With the exception of Rohrig (1977), in other studies the nutrient ions were measured after the prepruning root:shoot ratios were restored. After the prepruning root:shoot ratios are restored, the pruning stress is no longer operational as demonstrated by transpiration (Stansell et al., 1974; Taylor and Ferree, 1981) and photosynthesis (Abod et al., 1979; Detling et al., 1980; McDavid et al., 1973) studies. Thus, it seems that root pruning studies should be evaluated soon before the reestablishment of the prepruned functional equilibrium.

Rodney et al. (1956) demonstrated that low starch in roots was related to high Na in fibrous roots. Reduced concentrations of K, Cl, and Na were observed in roots infected with T. semipenetrans (Labanauskas et al., 1965) or inoculated with mycorrhiza (Graham and Syvertsen, 1989; Hartmond et al., 1987) compared to roots of control plants. However, the relation between root pruning and Cl and Na accumulation in leaves has not been studied.

Diurnal fluctuations in humidity may affect status of certain nutrient ions in leaves. For instance, concentrations of foliar Ca and K were low under high humidity regardless of whether the stress was imposed during the day or at night (Adams, 1991).

CHAPTER 3

LEACHING SOLUBLE SALTS INCREASES POPULATION DENSITIES OF *TYLENCHULUS SEMIPENETRANS*

Introduction

Worldwide, agricultural production is confronted with increasing levels of salinity levels in soil solution; whereas the cost of managing accumulated salt is also increasing (Nabors, 1984). Salinity generally decreases population densities of nematodes on some annual crops (Edongali et al., 1982; Heald and Heilman, 1971). Machmer (1958); however, found that salinity can increase population densities of the citrus nematode, *Tylenchulus semipenetrans* Cobb, on citrus under field conditions. The infectivity of *T. semipenetrans* juveniles after being in fallow soil at osmotic potential (π) levels ranging from -0.18 to -9.36 MPa did not differ from those of control nematodes (Kirkpatrick and Van Gundy, 1966). However, juvenile motility of *T. semipenetrans* was inhibited by π levels from -4.64 to -22.57 MPa (Kirkpatrick and Van Gundy, 1966). *In vitro* and in fallow soils, similar salt levels inhibited juvenile eclosion of *T. semipenetrans* (Appendix 1). Similar effects were observed for other four plant-parasitic nematodes (Dropkin et al., 1958).

Field observations in Israel (Cohn et al., 1965) and South Africa (Cohn, 1976) indicated that the highest densities of T. semipenetrans occur in citrus-producing areas with salinity. The conditions whereby salinity increases population densities of T. semipenetrans have not been studied. The objectives of this study were to determine whether salinity increases population densities of the citrus nematode through direct salt stress on nematodes, indirect salt stress in plants, or both. Since cation exchange capacity is dependent upon soil type and it influences salinity of the soil solution (Bohn et al., 1985), the effects of three soil types on salinity-nematode interactions were also investigated.

Materials and Methods

Salt tolerant Rangpur lime (Citrus reticulata var. austera Swingle) seeds were germinated in sand, and uniform 3-month-old seedlings were transplanted into 10-cm-diam clay pots containing steamed autoclaved loamy sand (82% sand, 5% silt, 13% clay; pH 6.9, 0.2% organic matter), sand (97% sand, 2% silt, 1% clay; pH 7.1, 0.1% organic matter), or organic mix 1:1 (v/v) sand:PRO-MIX BX (Premier Brands, Inc., Stamford, Canada). Salinity treatments were discontinuous salt (DS), continuous salt (CS), and no salt (NS) for each soil type. Pots were arranged in the greenhouse in a complete 3 x 3

factorial block design with nine replications. Ambient temperatures averaged 30 C maximum and 25 C minimum. Plants were irrigated with 100 ml tap water every other day and fertilized weekly with 100 ml solution of 3 g of a 20:20:20 ($N:P_2O_5:K_2O$) mixture per liter water.

Each pot was infested 2 days after transplanting with 10 ml supernatant of greenhouse cultured Glomus intraradices Schenck and Smith (Duke et al., 1986) prepared by blending 2 g roots of infected Sudangrass, Sorghum sudanense Stapf, sieving (150-μm-pore sieve), and diluting to 500 ml with water. Salts were added to the irrigation water 2 weeks after transplanting, first daily at 25 mols $NaCl/m^3$ H_2O + 3.3 mols $CaCl_2/m^3$ H_2O in 100 ml solution for 3 days and then every other day at 50 mols $NaCl/m^3$ H_2O + 6.6 mols $CaCl_2/m^3$ H_2O for 1 week. The soil for DS and NS treatments was leached 10 days after initiating salt treatment by irrigation with 250 ml water daily for 3 days. The soil for CS treatments was leached with 250 ml of 25 mols $NaCl/m^3$ H_2O + 3.3 mols $CaCl_2/m^3$ H_2O solution. Leachates were collected 1 day before leaching, 9 and 33 days after leaching. Electrical conductivity (Ec) of leachates was determined using the Ec meter and converted to π values (Bohn et al., 1985).

The nematode inoculum was prepared 1 week after leaching. Citrus roots infected with T. semipenetrans were collected from the field, placed in a 2-liter plastic bag half-filled with water, vigorously shaken, and the contents were passed

through a 150-μm-pore sieve nested on a 25-μm-pore sieve. The contents of the 25-μm-pore sieve were aerated in 4.5-liter water to keep the nematode juveniles in suspension while allowing eggs, soil particles, and some debris to settle. The suspension was passed through a 150-μm-pore sieve nested on a 25-μm-pore sieve and the contents of the latter were collected for inoculum. Plants were inoculated three times using a 10 ml glass syringe by placing nematodes in four 5-cm-deep holes in the soil around each plant at two-day intervals to give a total of Ca. 84,000 juveniles/plant.

At harvest, 45 days after initiating salt treatment, shoots were cut at surface soil and weighed. The pot contents were emptied, roots collected and weighed. Nematodes were separated from 1 g roots/plant by maceration and blending for 30 seconds in 10% NaOCl and passed through a 150-μm-pore sieve onto a 25-μm-pore sieve. The contents of the latter were washed into 96 ml glass tubes. After 12 hours to allow nematodes to settle, the tubes were standardized to 25-ml volume. Five drops of acid-fuschin stain were added to each tube and the contents were brought to a boil. Eggs, juveniles, and adults were counted from a 5-ml aliquot. All roots and fully developed leaves were dried at 70 C for 48 hours and powdered separately in a porcelain mortar. Chloride concentration of leaves and roots, used as index of plant stress, were measured by a Haake Chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ). Nematode data were

transformed to ln(x+1) prior to analysis of variance to homogenize the variance (Little and Hills, 1975). All data were analyzed using three-way analysis of variance. The degrees of freedom and their associated sum of squares were partitioned to determine the relative contributions of each factor to mean total treatment variations observed (Johnson and Berger, 1982; Little, 1981).

The experiment was repeated once using salt-sensitive Sweet lime (C. limettioides Tan.) on organic mix only and the three salinity treatments under the conditions and procedures described for Rangpur lime. Each treatment (DS, CS, NS) was replicated 15 times, and pots were arranged in a complete randomized-block design. The methods used were similar to those used for Rangpur lime, except that 4-month-old Sweet lime seedlings were inoculated twice at 2-day interval with a total of Ca. 73,000 juveniles/plant. Data were analyzed by two-way analyses of variance and means were compared by Duncan's multiple-range test. Unless stated otherwise, only significant ($P \leq 0.05$) F-statistics and treatments were not significant at $P \leq 0.10$.

Results

Mean nematode female densities per gram of root weight were the highest in the DS treatments and in the organic mix relative to other salt and soil treatments, respectively

(Table 3-1). Nematode female densities on plants grown in loamy sand were also higher than those on plants grown in sand. Mean female densities were not different between NS and CS treatments. Using partitioning of the degrees of freedom and their associated sum of squares (Little, 1981), salinity, soil type, and interaction contributed, respectively, 52%, 36%, and 12% ($\underline{P} \leq 0.10$) of the total treatment variation (TTV) in mean female densities. Mean juvenile densities were not correlated with egg densities, suggesting that at least some juveniles were the remnants from inocula (data not shown). Nematodes in DS treatments produced the most eggs; whereas, those in CS and NS treatments were not different. Nematodes in the organic mix also produced the most eggs followed by those in loamy sand and sand. The major sources of variation in mean egg counts were 83% for salinity and 14% ($\underline{P} \leq 0.10$) for soil type. Fecundity was expressed as number of eggs/female. Females in DS treatments had the highest fecundity; whereas, those in NS and CS treatments were not different ($P \leq 0.10$). The only source of treatment variation in mean fecundity was salinity.

Salinity accounted for over 97% of the TTV to mean π variations throughout the study with small contributions from soil type and interactions. Leachates from the CS treatments had the highest mean π for all sampling dates; whereas, mean

TABLE 3-1. *Tylenchulus semipenetrans* female counts per gram of fresh roots on salt-tolerant Rangpur lime as affected by soil type, discontinuous salt, continuous salt, or no salt treatments.

Salt treatment	Soil treatment: Loamy soil	Organic mix	Sand
No salt	75	121	32
Discontinuous salt	217	588	94
Continuous salt	57	123	45

Analysis of Variance

Source of variation	Total Treatment Variation: df	SS	Percentage
Salinity	2	103.91 **	52.00
Soil type	2	71.94 **	36.00
Salinity x soil	4	23.98 †	12.00
Error	72	168.12	

Each value is an average of 9 replicates.
** Significant at $\underline{P} \leq 0.01$, † $\underline{P} \leq 0.10$.
Sand: PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

π of DS and NS treatments, or that of loamy sand and sand, were not different (Table 3-2). Organic mix had the highest mean pH; whereas, those of loamy sand and sand were not different. The strongest source of variation in mean pH

TABLE 3-2. *Tylenchulus semipenetrans* egg counts per gram fresh roots on salt-tolerant Rangpur lime as affected by soil type, discontinuous salt, continuous salt, or no salt treatments.

Salt treatment	Soil treatment		
	Loamy soil	Organic mix	Sand
No salt	13	19	31
Discontinuous salt	363	533	159
Continuous salt	13	69	24

Analysis of Variance

Source of variation	Total Treatment Variation		
	df	SS	Percentage
Salinity	2	240.21 **	82.9
Soil type	2	8.84 ns	3.0
Salinity x soil	4	40.74 †	14.1
Error	72	330.55	

Each value is an average of 9 replicates.

** Significant at $P \leq 0.01$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

Sand: PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

throughout the study was soil type. There was no evidence of treatment effects on either fresh shoot or root weights (data not shown). Mean leaf Cl contents were highest in CS (1.4% Cl), moderately higher in DS (0.5% Cl), and low in NS (0.2%

TABLE 3-3. Fecundity of Tylenchulus semipenetrans females per gram fresh roots on salt-tolerant Rangpur lime as affected by soil type, discontinuous salt, continuous salt, or no salt treatments.

Salt treatment	Soil treatment: Loamy soil	Organic mix	Sand
No salt	0.2	0.2	1.0
Discontinuous salt	1.7	0.9	1.7
Continuous salt	0.2	0.6	0.5

Analysis of Variance

Source of variation	df	Total Treatment Variation: SS	Percentage
Salinity	2	67.94 †	63.94
Soil type	2	12.48 ns	11.75
Salinity x soil	4	25.83 ns	24.31
Error	72	106.25	

Each value is an average of 9 replicates.

† Significant at $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

Sand: PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

Cl) treatments. Salinity and salinity x soil interactions contributed 94% and 4% ($P \leq 0.10$), respectively, of the TTV in mean leaf Cl levels. Mean root Cl levels across all treatments or soil types were not different ($P \leq 0.10$).

TABLE 3-4. Osmotic potential (π) and pH of soil leachate as affected by soil type (loamy sand, organic mix, sand) and discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

Sampling	Soil	π ($-1x10^{-2}$ MPa)				pH			
time†	type	NS	DS	CS	Mean	NS	DS	CS	Mean
1	Loam	8	24	24	19a	6.6	7.1	6.6	7.0b
	Organic	7	27	23	19a	7.2	7.9	7.4	7.5a
	Sand	8	23	24	18a	6.9	7.7	6.9	7.2b
	Mean	8b	25a	24a		6.9b	7.6a	7.0b	
2	Loam	7	7	21	12a	6.9	7.0	6.8	6.9b
	Organic	7	7	23	13a	7.3	7.4	7.3	7.3a
	Sand	6	7	20	11a	7.3	6.8	6.9	7.0b
	Mean	7b	7b	22a		7.2a	7.1a	7.0a	
3	Loam	7	7	40	19a	5.9	6.0	5.6	5.8b
	Organic	8	9	37	18a	6.8	6.7	6.8	6.8a
	Sand	7	7	37	17a	5.6	5.3	5.9	5.6b
	Mean	8b	8b	38a		6.1a	60a	6.1a	

Means (n = 9) followed by the same letter within a column or row for each variable are not different ($\underline{P} \leq 0.05$) according to Duncan's multiple-range test.

† 1 = one day before leaching; 2 = 9 days after leaching; 3 = 33 days after leaching.

Organic mix = Sand:PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

TABLE 3-5. Tylenchulus semipenetrans female and egg counts per gram of fresh roots on salt-sensitive Sweet lime as affected by discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

	Salt treatment		
Variable	DS	CS	NS
Females	101.0a	28.0b	21.0b
Eggs	386.0a	15.0b	8.0b
Fecundity	3.8a	0.5b	0.4b

Column means (n = 15) with the same letter are not different ($\underline{P} \leq 0.05$) according to Duncan's multiple-range test.

Numbers of T. semipenetrans females and eggs on Sweet lime in DS treatments were greater than those in CS and NS treatments, while in the latter they were not different (Table 3). Mean juvenile numbers were neither different among treatments nor correlated with numbers of eggs (data not shown). Fecundity was higher in the DS treatment than in other treatments and was not different between the CS and NS treatments.

The effects of soil salinity on π or pH for Sweet lime were similar to those for Rangpur lime on organic mix (Table 4). Salinized plants had higher leaf Cl levels than controls on all sampling dates. Root Cl levels in all treatments were not different (data not shown).

TABLE 3-6. Osmotic potential (π) and pH of soil leachate, and leaf chloride (Cl) of Sweet lime as affected by soil type (loamy sand, organic mix, sand) and discontinuous salt (DS), continuous salt (CS), or no salt (NS) treatments.

Sampling time†	Salt treatment	Soil π ($-1x10^{-2}$ MPa)	Soil pH	Leaf Cl (%)
1	DS	24a	8.0a	0.76a
	CS	21a	7.3a	0.61a
	NS	3b	7.7a	0.07b
2	DS	8b	7.7a	0.31b
	CS	30a	6.9ab	1.58a
	NS	7b	6.3b	0.10c
3	DS	10b	6.0b	0.27b
	CS	40a	7.1a	1.69a
	NS	8b	5.8b	0.09c

Column means (n = 9) with the same letter are not different ($\underline{P} \leq 0.05$) according to Duncan's multiple-range test.

† 1 = one day before leaching; 2 = 9 days after leaching; 3 = 33 days after leaching.

Organic mix = Sand:PRO-MIX BX (1:1, v/v), Premier Brands, Inc.

Discussion

Discontinuous salt is similar to irrigation with poor quality water under field conditions where rainfall can leach salt from the soil profile. Although the CS treatment did not effect populations of T. semipenetrans, DS treatment had a tremendous influence. Tylenchulus semipenetrans in the DS treatments was not exposed to continuous salt stress in the soil environment which inhibits nematode movement (Kirkpatrick and Van Gundy, 1966; Lee and Atkinson, 1977). In other studies, the CS levels decreased population densities of Meloidogyne incognita on tomato (Edongali et al., 1982) and had no effect on populations of Rotylenchulus reniformis Linford and Oliveira on cotton (Heald and Heilman, 1971). Results in this study suggest that temporary salt stress on the plant predisposes the host to T. semipenetrans infection only in the absence of osmotic stress in soil solution.

In the DS or NS treatments the mean π was less than -1.44 MPa and the pH was less than 8.5, which are considered to be the upper limits of non-saline soils (Bohn et al., 1985; Sposito, 1989). In the CS treatment, the mean π was greater than -1.44 MPa and the pH was less than 8.5, meeting the criteria for salinity affected soils. This confirms the ease with which leaching can convert saline to normal soil under suitable conditions (Bohn et al., 1985). The high pH in organic mix was probably due to the high cation exchange

capacity of the soil (Bohn et al., 1985). The mean pH range 6.0 - 8.0 was within the optimum ranges for T. semipenetrans population development (Duncan and Cohn, 1990). The reason for higher pH in DS prior to leaching is unknown. The lowest and the highest nematode population densities, respectively, in the sandy soil and the organic mix in Rangpur lime confirmed earlier findings (O'Bannon, 1968).

Plants in CS treatments had leaf Cl levels above the mean toxic level of 1% (Smith, 1966); but over the short duration of this study, there was no noticeable defoliation. In both experiments, plants in DS treatments had higher leaf Cl content than the controls. These results suggest the inability of either rootstock to reduce Cl accumulation in shoots even after leaching salts from the root zone. Mean leaf Cl levels in the DS treatments were higher than the physiological damage threshold of 0.20% (Smith, 1966), suggesting that the plants were salt-stressed for the duration of the study. Roots or leaves in NS treatments had higher Cl levels than usually reported in NS control plants (Zekri, 1987). It was previously shown that G. intraradices increases Cl levels in citrus (Graham and Syvertsen, 1989). That may partly account for the higher Cl levels in NS plants in this study. However, because the magnitudes of Cl levels in our NS plants were higher than those in G. intraradices infected plants (Graham and Syvertsen, 1989), and because all the NS plants were also infected with the citrus nematode, it seems

that this parasite may also be increasing the Cl in citrus leaves. Because Machmer's (1958) studies were conducted under field conditions over three years, it is conceivable that rainfall occasionally leached salts, creating conditions similar to those in these studies. Periodic salinity and rainfall leaching similarly may account for the higher population densities of _T. semipenetrans_ observed in most citrus-producing areas with salinity (Cohn, 1976; Cohn et al., 1965). This study projects increasing _T. semipenetrans_ problems in citrus-producing areas because (1) NaCl salt concentrations of irrigation water in citrus producing areas are increasing, (2) salt leaching, which increases population densities of _T. semipenetrans_, is the major strategy of controlling salinity in the root zone, and (3) salinity accentuates the severity of the citrus nematode damage.

CHAPTER 4
SALINITY REDUCES RESISTANCE TO TYLENCHULUS SEMIPENETRANS IN CITRUS ROOTSTOCK SEEDLINGS

Introduction

Nematode-resistant rootstocks play a major role in integrated management of the citrus nematode, *Tylenchulus semipenetrans* Cobb (Garabedian et al., 1984; Kaplan, 1988). However, there is currently no commercial citrus rootstock that is both tolerant to salinity and resistant to *T. semipenetrans* (Castle et al., 1989; Newcomb, 1978).

Worldwide, salt concentrations of irrigation water in major citrus-producing regions are increasing (Bielorai et al., 1988; Nieves et al., 1992; Shalhevet et al., 1974; Syvertsen et al., 1989). Depending on root condition, tree age, and soil type, high population densities of *T. semipenetrans* usually occur in areas with salinity (Cohn, 1976; Machmer, 1958). Recently (Chapter 3), it was demonstrated that leaching soluble salts after a short period of salinity stress increases infection of *T. semipenetrans* on nematode susceptible citrus rootstocks.

Salinity (Bielorai et al., 1988; Shalhevet et al., 1974) and *T. semipenetrans* (Cohn, 1972) can each reduce citrus growth and yield. The effect of salinity on the expression of

host resistance to *T. semipenetrans* has not been studied. The objectives of this research were to test the effects of salinity on host resistance to *T. semipenetrans* in citrus rootstock seedlings representing a wide range of *T. semipenetrans*-resistant germplasm.

Materials and Methods

Six citrus rootstocks were selected to represent a wide range of *T. semipenetrans*-resistant germplasm. Highly resistant rootstocks were trifoliate orange (*Poncirus trifoliata*) and Swingle citrumelo (*Citrus paradisi* x *P. trifoliata*). Moderately resistant rootstocks were Carrizo citrange (*C. sinensis* x *P. trifoliata*) and Troyer citrange (*C. sinensis* x *P. trifoliata*). Highly susceptible rootstocks were Cleopatra mandarin (*C. reticulata*) and sour orange (*C. aurantium*). Seedlings of each rootstock were raised in plywood boxes containing a potting mix consisting of three volumetric parts of sandy soil (97% sand, 2% silt, 1% clay; 2% organic matter) and one part organic supplement PRO-MIX BX (Premier Brands, Inc., Stamford, Canada). Seedlings were inoculated with a suspension of *Glomus intraradices* Schenck and Smith 2 months after emergence, prepared as previously described (Chapter 3). Seedlings in boxes were irrigated twice weekly, and fertilized once weekly with 25% Hoagland's solution (Hoagland and Arnon, 1950).

Seedlings were selected for uniformity 4 months after emergence and transplanted individually into 15-cm-diam clay pots containing the described potting mix. Seventeen replications of each rootstock with and without salt treatment were arranged in a greenhouse in a randomized complete-block split plot design. Transplants were irrigated with 150 ml tap water every other day and fertilized weekly with 150 ml solution of 5 g of a 20:20:20 ($N:P_2O_5:K_2O$) mixture per liter water and monthly with 25% Hoagland's solution to provide micronutrients. To achieve temporary saline conditions in the potting mix, irrigation water for half of the plants per rootstock species was supplemented with NaCl and $CaCl_2$ for a 3-week period beginning 2 months after transplanting. The concentrations (moles/m^3) of NaCl and $CaCl_2$ were, respectively, 17 and 3 the first week, 50 and 8.8 the second week, and 100 and 17.6 the third week. Thus, salt concentrations were increased gradually and the total amounts of salt applied were 167 moles NaCl and 29.4 moles $CaCl_2$ per cubic meter water. The other half of the plants per rootstock species served as salt-free controls. Non-saline conditions were recreated by leaching salt-treated soil with 300 ml tap water at 2-day interval for 1 week at the end of the 3-week salinization period. Salt-free controls were also leached. Leachate was collected from each plant at final leaching and mean electrical conductivity was verified to be 0.806 dS/m, which converts to an osmotic potential of -0.290 MPa (Bohn et al.,

1985). Salt leaching prior to nematode inoculation was designed to simulate field conditions, to predispose host roots to infection, and to avoid direct adverse osmotic potential on nematodes.

Nematode inoculum was collected, prepared, and each seedling was inoculated four times at 3-day interval to obtain a total of ca. 73,000 nematodes/ plant, beginning 3 days after the final leaching as described previously (Chapter 3). Ambient greenhouse temperatures from inoculation to harvest averaged 31 C maximum (range 28-32) and 25 C (range 23-26).

At harvest, 8 weeks after the initial inoculation, nematodes were extracted by macerating 2 g fresh roots per plant, prepared and counted as described previously (Chapter 3). Female fecundity (the number of eggs plus juveniles per female) was calculated for each treatment. Total fresh fibrous roots of each plant were weighed. Also, shoots, fibrous roots, and tap roots were weighed after drying at 70 C for 48 hours.

Treatment effects were evaluated using analysis of variance (ANOVA) without the block factor, which analysis indicated was not significant ($\underline{P} \leq 0.10$). The degrees of freedom and their associated sum of squares were partitioned to determine the percentage contribution of main factors and interactions to the total treatment variations (TTV) among the treatment means (Little, 1981). The nematode data were transformed to ln(x+1) prior to ANOVA to homogenize the

variance (Little and Hills, 1975), but untransformed data are reported. Unless stated otherwise, only treatments where the sum of squares were significant ($\underline{P} \leq 0.05$) are discussed.

Results

Salinity increased nematode female development and reproduction on all rootstock species, but nematode densities in resistant rootstocks were consistently lower than those in susceptible rootstocks. The partitioning the degrees of freedom and their associated sums of squares (Little, 1981), demonstrated that rootstock treatment contributed 95%, 64%, and 86% to total treatment variation (TTV) in female development (Table 4-1), juvenile (Table 4-2), and egg (Table 4-3), respectively; whereas salinity 3%, 22%, and 24%. There were no rootstock x salinity interactions except for a small effect (2%, $\underline{P} \leq 0.10$) on mean female counts. Mean separation of nematode densities among the rootstocks generally followed the degree of nematode resistance on the rootstocks. Only salinity effect contributed (42%) to TTV in mean female fecundity (Table 4-4).

Sour orange had the highest mean fresh fibrous root weight and trifoliate orange had the lowest; whereas those of Cleopatra mandarin, Swingle citrumelo, Carrizo and Troyer citranges were intermediate under both salt-free controls

TABLE 4-1. _Tylenchulus semipenetrans_ female counts per gram of fresh roots 8 weeks after inoculations of highly resistant (R), moderately resistant (M), and susceptible (S) citrus rootstock seedlings previously grown with and without salinity.

		Salt treatment	
Rootstock	Class	Control	Salinity
Sour orange	S	162	193
Cleopatra mandarin	S	245	270
Carrizo citrange	M	45	49
Troyer citrange	M	29	41
Swingle citrumelo	R	4	15
Trifoliate orange	R	9	17

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	34.14 **	95.00
Salinity	1	1.08 *	3.00
R x S	5	0.72 ns	2.00
Error	105		

Each value is an average of 15 replicates.
** Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

TABLE 4-2. *Tylenchulus semipenetrans* juvenile counts per gram of fresh roots 8 weeks after inoculations of highly resistant (R), moderately resistant (M), and susceptible (S) citrus rootstock seedlings previously grown with and without salinity.

		Salt treatment	
Rootstock	Class	Control	Salinity
Sour orange	S	49	73
Cleopatra mandarin	S	112	88
Carrizo citrange	M	52	73
Troyer citrange	M	56	74
Swingle citrumelo	R	26	53
Trifoliate orange	R	30	52

Analysis of Variance			
Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	23.96 **	69.10
Salinity	1	4.58 *	13.21
R x S	5	6.13 ns	17.69
Error	105	34.67	

Each value is an average of 15 replicates.

** Significant at $P \leq 0.01$, * $P \leq 0.05$; ns = not significant at $P \leq 0.1$.

TABLE 4-3. *Tylenchulus semipenetrans* egg counts per gram of fresh roots 8 weeks after inoculations of highly resistant (R), moderately resistant (M), and susceptible (S) citrus rootstock seedlings previously grown with and without salinity.

		Salt treatment	
Rootstock	Class	Control	Salinity
Sour orange	S	711	1,283
Cleopatra mandarin	S	704	1,406
Carrizo citrange	M	156	547
Troyer citrange	M	93	169
Swingle citrumelo	R	23	227
Trifoliate orange	R	46	92

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	310.96 **	86.78
Salinity	1	26.68 **	7.44
R x S	5	20.71 ns	5.78
Error	108	255.35	

Each value is an average of 15 replicates.

** Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

TABLE 4-4. Fecundity (number of eggs/female) of _Tylenchulus semipenetrans_ females 8 weeks after inoculations of highly resistant (R), moderately resistant (M), and susceptible (S) citrus rootstock seedlings previously grown with and without salinity.

		Salt treatment	
Rootstock	Class	Control	Salinity
Sour orange	S	2.56	3.03
Cleopatra mandarin	S	2.53	2.45
Carrizo citrange	M	2.21	3.02
Troyer citrange	M	2.17	2.73
Swingle citrumelo	R	1.77	2.87
Trifoliate orange	R	2.94	3.00

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	5.66 ns	31.93
Salinity	1	7.49 **	42.19
R x S	5	4.59 ns	25.88
Error	108	5.84	

Each is an average of 15 replicates.
** Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

TABLE 4-5. Root and shoot weights (g) of 9-month-old highly resistant (R), moderately resistant (M), and susceptible (S) citrus rootstock seedlings that were exposed to a 3-week salt treatment (salt) or not exposed (control) when 6 months old and then inoculated with Tylenchulus semipenetrans when 7 months old.

		Fibrous roots			
		Fresh		Dry	
Rootstock	Class	Control	Salt	Control	Salt
Sour orange	S	6.5a	6.0a	1.0a	0.9a
Cleopatra mandarin	S	3.1b	3.7b	0.3b	0.4b
Carrizo citrange	M	3.1b	2.9b	0.3b	0.3b
Troyer citrange	M	2.7b	2.7bc	0.3b	0.3b
Swingle citrumelo	R	3.5b	3.1b	0.6ab	0.5b
Trifoliate orange	R	1.7c	1.5c	0.3b	0.4b
		Dry tap root		Dry shoot	
		Control	Salt	Control	Salt
Sour orange	S	1.6a	1.8a	5.4a	4.5a
Cleopatra mandarin	S	0.5b	0.5b	2.4c	2.7c
Carrizo citrange	M	1.0ab	0.7b	2.4c	2.7c
Troyer citrange	M	0.8b	0.7b	2.4c	2.7c
Swingle citrumelo	R	1.1ab	0.8b	4.2b	3.0b
Trifoliate orange	R	0.7b	0.5b	1.8d	1.4d

Column means ($n = 17$) with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

and salt treatment (Table 4-5). Only the rootstock effect contributed (98%) to TTV in mean fresh fibrous root weights. Similar trends were observed in dry fibrous roots. Sour orange and Cleopatra mandarin had, respectively, the highest and lowest mean total dry root weights; whereas the intermediate weights were not different. Rootstock contributed 79% and salinity 13% to TTV in mean dry tap root weights, with no evidence of interaction effect. Rootstock contributed 89% and salinity 5% to TTV in mean dry total root weights, with no evidence of interaction effect. There was no salinity effect on top weights.

Discussion

Inherent differences (Castle et al., 1989) in rootstocks were the major source of variation in root weights. Salinity had no measurable effect on fresh or dry fibrous root weight but did cause small (ca. 20%) decreases in dry tap and total root weights. Thus, the primary effect of salinity on the root system was the reduction of tap root growth. The role of citrus tap root in salt tolerance is not clear, since tap roots generally contain lower Cl than fibrous roots (Grieve and Walker, 1983). Tylenchulus semipenetrans primarily feeds on fibrous roots (Cohn, 1972).

The most notable effect of salinity was to increase nematode egg production by several fold in all rootstocks.

Citrus resistance to *T. semipenetrans* is expressed as suppression of female development to maturity (Kaplan, 1988; Van Gundy and Kirkpatrick, 1964). Development to maturity, even in resistant rootstocks, invariably leads to egg production (Kaplan, 1981). Females on Swingle citrumelo, the most widely used and the most salt-sensitive citrus rootstock (Castle et al., 1989), had the greatest relative increase in egg production (10-fold) due to salt treatment. Swingle citrumelo and trifoliate orange possess differential resistance (Kaplan, 1981), which often is readily overcome by pathogens, including plant-parasitic nematodes (Fry, 1982; Triantaphyllou, 1987). The enhanced female development and increased fecundity due to salt stress on the host may eventually increase the selection pressure against resistant genes. Biotypes of *T. semipenetrans* capable of reproducing prolifically in resistant trifoliate orange rootstocks, have in fact, been reported from citrus producing regions with salinity (Gottlieb et al., 1986; Inserra et al., 1980).

Generally, under citrus orchards salinity is a seasonal problem. Salts accumulate in the rhizosphere during extended irrigation seasons and leach from the rhizosphere during rainy seasons (Bielorai et al., 1988; Syvertsen et al., 1989). Results of this and the previous studies (Chapter 3) suggest that salt accumulation and leaching cycles can augment *T. semipenetrans* populations even in resistant rootstocks, and

may also explain higher population densities of this nematode in areas with salinity (Cohn, 1976; Machmer, 1958).

Since salinity increased nematode development and fecundity in all citrus rootstocks tested, and since all nematode resistant rootstocks lack salt tolerance (Castle et al., 1989; Newcomb, 1978), increasing salinity in irrigation water affects both citrus breeding and nematode management. As in cereal crops (Nabors, 1984), salt tolerant genes should be incorporated into multiple resistance rootstocks such as Carrizo and Troyer citranges. Alternatively, nematode resistance genes could be introduced into the salt tolerant (Maas, 1993) rootstocks.

CHAPTER 5
TYLENCHULUS SEMIPENETRANS REDUCES SALT TOLERANCE IN CITRUS ROOTSTOCK SEEDLINGS

Introduction

The continuous increase of NaCl salinity in irrigation water (Bielorai et al., 1988; Bohn et al., 1985; Chapman, 1968; Nabors, 1984; Syvertsen et al., 1989; Waisel, 1972) suggests that salt-tolerant rootstocks may be integral in future management of Cl and (or) Na toxicities in citrus. Salt tolerance in citrus is defined as the ability of roots to exclude Cl and (or) Na from shoots (Castle et al., 1989). All commercial salt-tolerant citrus rootstocks are susceptible to the citrus nematode, *Tylenchulus semipenetrans* Cobb (Castle et al., 1989). Van Gundy and Martin (1961) found that this nematode caused an increase in Na in leaves of salt-sensitive sweet orange seedlings. The effects of *T. semipenetrans* parasitism of roots on salt tolerance in salt-tolerant rootstocks have not been studied. The objectives of this research were to measure the effects of *T. semipenetrans* infection on salt tolerance in citrus rootstock seedlings with a wide range of salt tolerance using low saline and nonsaline irrigation water. Because ion absorption and exclusion require metabolic energy (Epstein, 1972; Marschner, 1986;

Waisel, 1972), the nonstructural carbohydrates in both leaves and roots were measured to enhance the relation between this variable and ionic accumulation.

Materials and Methods

Citrus rootstocks (Castle et al., 1989; Maas, 1993) studied were salt-tolerant Cleopatra mandarin (*Citrus reticulata* Blanco) and Rangpur lime (*C. limon* Osbeck), moderately salt-tolerant sour orange (*C. aurantium* L.) and rough lemon (*C. limon*), and salt sensitive Sweet lime (*C. aurantifolia* Tanaka) and Volkamer lemon (*C. volkameriana* Tanaka). Seeds of each rootstock were planted in two plywood boxes, 55 x 34 x 25 cm, containing a potting mix of 3:1 (v/v) steamed autoclaved sand (97% sand, 2% silt, 1% clay; pH 7.1, 0.2% organic matter) and organic supplement PRO-MIX BX (Premier Brands, Inc., Stamford, Canada). All seedlings were infested 2 months after emergence with the vesicular-arbuscular mycorrhiza, *Glomus intraradices* Schenck & Smith (Harley and Smith, 1983), collected and prepared as described previously (Chapter 3). The nematode inoculum was collected beginning 4 months after seedling emergence, prepared and placed in the soil around roots of one-half of the seedlings of each species as described previously (Chapter 3). Each seedling was inoculated with a total of ca. 90,000 nematode juveniles at weekly intervals for 6

weeks. Nematode-free control seedlings were inoculated with nematode inoculum filtrate (25-μm-pore sieve) to establish in their rhizosphere any microbes associated with the nematode.

All seedlings were initially irrigated with tap water having electrical conductivity (Ec) 0.357 dS/m at 4-day intervals, and fertilized with 25% Hoagland's solution (Hoagland and Arnon, 1950) weekly. Seedlings were selected for uniformity 3 months after initial inoculation with nematodes and transplanted into 15-cm-diam clay pots containing the previously described potting mix. Each nematode-treated transplant was reinoculated at 3-day interval for 2 weeks with a total of ca. 96,000 nematodes to insure that new roots in the potting mix were infected. Transplants were irrigated with 150 ml tap water every other day and fertilized weekly with 200 ml solution of 5 g of a 20:20:20 ($N:P_2O_5:K_2O$) mixture per liter water and biweekly with 200 ml of 25% Hoagland's solution as a source of micronutrients. To achieve saline conditions in the soil, irrigation water for one-half of the nematode-treated and nematode-free control plants per rootstock species was supplemented with 17 mols $NaCl/m^3$ H_2O + 3 mols $CaCl_2$ $/m^3$ H_2O (Ec = 2.230 dS/m) for 4 weeks beginning 3 months after transplanting. Calcium chloride was included as a source of Ca, which is essential for the maintenance of cell membranes, particularly under saline conditions (Maas,

1993). Pots were arranged in the greenhouse in a randomized, complete block factorial design with 15 replications.

Three fully developed leaves/plant were sampled, and dried at 70 C for 48 hours, and leaves were ground in a Wiley mill to pass a 375-μm-pore sieve. The concentrations of Cl from 1 g ground leaf tissue were verified using a Haake Chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) after a 12-hour extraction in 1 N nitric-acetic acid (Rhue and Kidder, 1983). The leachate pH and Ec were verified 1 day before harvest at the end of the 4-week salinization period.

The shoots and roots were separated by cutting at the surface of the soil 4 weeks after salinization. Nematodes were extracted from a 1-g root sample, stained, and counted as previously described (Chapter 3). Shoots and the remaining nematode-infected and nematode-free roots were dried at 70 C for 48 hours and weighed. Roots and mature leaves were then ground separately, and 1 g of leaf and root tissue separately analyzed for Cl. One gram each of ground root and leaf tissues was ashed at 500 C for 6 hours, and the ash was dissolved in 20 ml 1 N HCl. The concentrations of Na, Ca, K, Mg, P, Cu, Fe, Mn, and Zn were measured from a 5-ml aliquot (Rhue and Kidder, 1983) by an inductively coupled plasma emission spectrometer (Perkin Elmer Co., Norwalk, CT). Soluble root and leaf carbohydrates of

Rangpur lime, sour orange, and Sweet lime were extracted by boiling 50 mg ground tissue for 2 minutes in 15 ml water followed by centrifugation (2,000 rpm) for 2 minutes. Glucose oxidase (Sigma) was used to analyze free glucose in the supernatant (Nelson, 1944). Soluble starch in the supernatant and insoluble starch in the pellet were analyzed with glucose oxidase (Smith, 1981) following 48 hours of amyloglucosidase (Sigma) digestion. Arsenomolybdate (Sigma) was used to analyze reducing sugars (Roe et al., 1949) and resorcinol reagent (Smith, 1981) to analyze ketone sugars.

Treatment effects were evaluated using analysis of variance (ANOVA) without the block factor, which analysis indicated was not significant ($\underline{P} \leq 0.10$). The degrees of freedom and their associated sum of squares were partitioned to determine the percentage contribution of main factors and interactions to the total treatment variations (TTV) among treatment means (Little and Hills, 1978). Insoluble and soluble starch data were combined prior to ANOVA. Nematode data were transformed to ln(x+1) prior to ANOVA to homogenize the variance (Little and Hills, 1978), but untransformed data are reported. Unless stated otherwise, only data where sum of squares were significant ($\underline{P} \leq 0.05$), and treatments were not significant at $\underline{P} \leq 0.10$.

Results

Relative to untreated controls, the _T_. _semipenetrans_, salinity, and rootstock treatments generally increased the accumulation of Cl and Na in leaves, and decreased the two ions in roots. The nematodes contributed 35%, salinity 21%, rootstock 9%, and salinity x nematode interaction 26% to the TTV in mean leaf Cl (Table 5-1), while salinity contributed 85%, nematodes 4%, rootstocks 2%, and salinity x nematode 3% to the TTV in mean root Cl (Table 5-2). Also, the nematodes contributed 28% and salinity 18% to the TTV in mean leaf Na (Table 5-3), while salinity contributed 71%, nematodes 10%, and the salinity x nematode interaction 10% to the TTV in mean root Na (Table 5-4).

The treatment effects were also consistent among all the rootstocks for K (Tables 5-5, 5-6). The rootstocks contributed 26%, nematodes 20%, salinity 16%, and rootstock x salinity interaction 22% ($\underline{P} \leq 0.10$) to the TTV in mean leaf K (Table 5-5). The nematodes contributed 60%, salinity 12%, rootstocks 5%, and rootstock x nematode interaction 20% to the TTV in mean root K (Table 5-6). The treatments also affected the concentrations of Cu (Appendix 3), Ca (Appendix 5), Mg (Appendix 7), Zn (Appendix 9), Mn (Appendix 11), and P (Appendix 13) in leaves, and Cu (Appendix 4), Ca (Appendix 6), Mg (Appendix 8), Zn (Appendix 10), Mn (Appendix 12), and

TABLE 5-1. Concentrations (% weight) of chloride in leaves of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	0.05	0.12	0.08	0.53
Rangpur	H	0.07	0.09	0.07	0.28
Sour orange	M	0.08	0.16	0.08	0.67
Rough lemon	M	0.21	0.20	0.08	0.93
Sweet lime	S	0.11	0.17	0.11	0.63
Volkamer	S	0.12	0.12	0.07	0.57

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	1.70 **	8.45
Salinity	1	4.13 **	20.53
Nematode	1	6.90 **	34.29
R x S	5	0.35 ns	1.74
R x N	5	0.76 *	3.78
S x N	1	5.40 **	26.84
R x S x N	5	0.88 *	4.37
Error	337	23.00	

Each value is an average of 15 replicates.
** Significant at ($P \leq 0.01$; * $P \leq 0.05$; ns = not significant at $P \leq 0.10$.

TABLE 5-2. Concentrations (% weight) of chloride in roots of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	0.17	0.11	1.12	0.84
Rangpur	H	0.45	0.08	0.87	0.73
Sour orange	M	0.20	0.15	0.99	0.97
Rough lemon	M	0.16	0.16	1.51	0.58
Sweet lime	S	0.13	0.14	1.20	0.88
Volkamer	S	0.12	0.11	0.91	0.72

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	1.27 **	1.86
Salinity	1	58.16 **	85.09
Nematode	1	2.65 **	3.88
R x S	5	0.54 ns	0.79
R x N	5	1.78 **	2.60
S x N	1	1.73 **	2.53
R x S x N	5	2.22 **	1.46
Error	337	44.95	

Each value is an average of 15 replicates.

** Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

TABLE 5-3. Concentrations (% weight) of sodium in leaves of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	0.17	0.35	0.20	0.48
Rangpur	H	0.17	0.21	0.19	0.41
Sour orange	M	0.11	0.27	0.24	0.34
Rough lemon	M	0.14	0.32	0.24	1.24
Sweet lime	S	0.11	0.69	0.19	0.69
Volkamer	S	0.14	0.38	0.18	0.38

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	1.55 **	12.65
Salinity	1	1.99 **	16.24
Nematode	1	3.10 **	24.31
R x S	5	1.31 ns	10.69
R x N	5	1.31 ns	10.69
S x N	1	1.88 **	15.35
R x S x N	5	1.11 ns	9.06
Error	168	29.16	

Each mean is an average of 8 replicates.

** Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

TABLE 5-4. Concentrations (% weight) of sodium in roots of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	0.50	0.14	0.33	0.23
Rangpur	H	0.49	0.13	0.34	0.11
Sour orange	M	0.64	0.17	0.45	0.19
Rough lemon	M	0.58	0.19	0.26	0.11
Sweet lime	S	0.51	0.19	0.39	0.09
Volkamer	S	0.60	0.14	0.26	0.14

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	0.14 ns	2.50
Salinity	1	3.98 **	70.94
Nematode	1	0.55 **	9.80
R x S	5	0.09 ns	1.60
R x N	5	0.18 ns	3.21
S x N	1	0.56 **	9.98
R x S x N	5	0.11 ns	1.96
Error	168	4.25	

Each value is an average of 8 replicates.
** Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

TABLE 5-5. Concentrations (% weight) of potassium in leaves of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	2.43	1.89	1.90	1.44
Rangpur	H	2.68	2.63	2.19	1.74
Sour orange	M	2.36	1.72	1.96	2.05
Rough lemon	M	2.00	1.76	2.08	1.76
Sweet lime	S	2.58	2.28	2.00	2.08
Volkamer	S	1.98	1.91	2.23	1.75

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	4.84 **	26.46
Salinity	1	2.98 **	16.29
Nematode	1	3.75 **	20.50
R x S	5	3.97 *	21.71
R x N	5	0.66 ns	3.61
S x N	1	0.02 ns	0.11
R x S x N	5	2.07 ns	11.32
Error	168	66.49	

Each value is an average of 8 replicates.

** Significant at $\underline{P} \leq 0.01$, * $\underline{P} \leq 0.05$; ns = not significant at $\underline{P} \leq 0.10$.

TABLE 5-6. Concentrations (% weight) of potassium in roots of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	1.91	1.27	1.63	0.68
Rangpur	H	1.82	1.32	1.64	1.30
Sour orange	M	2.07	1.20	1.46	0.98
Rough lemon	M	3.01	1.05	2.33	0.66
Sweet lime	S	2.11	1.68	1.52	1.22
Volkamer	S	2.39	1.00	1.93	0.84

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	3.20 **	5.14
Salinity	1	7.21 **	11.58
Nematode	1	37.60 **	60.40
R x S	5	1.05 **	1.69
R x N	5	12.29 ns	19.74
S x N	1	0.30 **	0.48
R x S x N	5	0.60 ns	0.96
Error	168	47.77	

Each value is an average of 8 replicates.

** Significant at $P \leq 0.01$; ns + not significant at $P \leq 0.10$.

TABLE 5-7. Concentrations (% weight) of starch in roots of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Rangpur	H	2.44	3.57	3.35	4.14
Sour orange	M	1.51	3.31	1.69	2.94
Sweet lime	S	1.75	3.63	2.55	4.18

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	2	13.07 **	20.13
Salinity	1	7.80 **	12.79
Nematode	1	35.04 **	53.94
R x S	2	9.10 **	14.17
R x N	2	0.13 ns	0.23
S x N	1	0.39 ns	0.58
R x S x N	2	0.13 ns	0.21
Error	72	39.25	

Each value is an average of 8 replicates.
** Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

TABLE 5-8. Concentrations (% weight) of ketone sugars in roots of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Rangpur	H	2.42	1.78	2.72	1.56
Sour orange	M	2.76	2.87	2.49	1.98
Sweet lime	S	2.52	2.09	2.68	2.46

Analysis of Variance

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	2	14.85 ns	18.74
Salinity	1	13.32 ns	16.81
Nematode	1	42.78 **	54.00
R x S	2	2.59 ns	3.27
R x N	2	4.22 ns	5.33
S x N	1	1.11 ns	1.40
R x S x N	2	0.34 ns	0.43
Error	72	40.56	

Each value is an average of 8 replicates.
** Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

TABLE 5-9. Mean shoot and root weights (g) of highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Shoot		Root	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	H	1.44	0.85	0.64	0.42
Rangpur	H	1.94	1.15	0.78	0.61
Sour orange	M	2.09	1.83	1.04	0.89
Rough lemon	M	3.31	0.86	1.20	0.36
Sweet lime	S	3.66	2.73	1.51	1.35
Volkamer	S	1.81	1.37	0.70	0.73

Analysis of Variance

		Total Treatment Variation			
		Shoot		Root	
variation	df	SS	Percentage	SS	Percentage
Rootstock	5	150.02 **	53.89	29.78 **	67.25
Salinity	1	0.17 ns	0.06	0.11 ns	0.25
Nematode	1	73.28 **	26.32	5.66 **	12.78
R x S	5	1.00 ns	0.36	1.41 +	3.18
R x N	5	48.27 **	17.34	6.77 **	15.29
S x N	1	0.47 ns	0.17	0.07 ns	0.16
R x S x N	5	5.16 ns	1.85	0.48 ns	1.08
Error	337	204.50		48.52	

Each value is an average of 15 replicates.

** Significant at $P \leq 0.01$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

TABLE 5-10. Tylenchulus semipenetrans on highly salt-tolerant (H), moderately salt-tolerant (M), and salt-sensitive (S) citrus seedling under nonsalinity and low salinity 4 weeks after salinity.

		Nematode densities/g fresh roots		
Rootstock	Class	Females	Juveniles	Eggs
Cleopatra	H	312ab	403a	6,633a
Rangpur	H	431a	613a	5,156a
Sour orange	M	262bc	432a	6,632a
Rough lemon	M	87c	112b	5,717a
Sweet lime	S	370a	373a	3,788a
Volkamer	S	270bc	278a	4,793a

Data pooled across salinity. Column means (n = 15) with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

P (Appendix 14) in roots. Micronutrient variations in both leaf and root tissues were mainly due to the rootstock differences. However, nematodes also contributed to the TTV of leaf Cu (31%) and root Mn (32%) and Zn (52%). Overall, T. semipenetrans had no effects on Mg, Fe, and Zn in leaves, or Cu and Fe in roots.

The treatments increased the concentrations of starch in roots of the three rootstocks tested (Table 5-7). However, the treatments reduced ketone sugars in the three rootstocks except sour orange under nonsalinity (Table 5-8). The nematodes accounted for 54%, salinity 12%, rootstocks

20%, and rootstock x nematode interaction 14% of the TTV in mean root starch. The nematodes reduced ketone sugars, and accounted for 54% of the TTV in mean ketone sugar concentrations. There were no treatment effects on all leaf carbohydrates tested or the reducing sugars in roots.

The rootstock and nematode treatments affected shoot and root growth (Table 5-9) without salinity effects (salinity data not shown). The nematodes suppressed shoot growth by an average of 64% (range 19-85%). Overall, the rootstocks contributed 54%, nematodes 26%, and rootstock x nematode interaction 17% ($\underline{P} \leq 0.05$) to the TTV in mean shoot weights. The nematode also limited mean root growth by 72% (range 25-92%). The rootstocks contributed 67%, nematodes 13%, rootstock x nematode interaction 15%, and rootstock x salinity interaction 3% to the TTV in mean root weights.

The nematode population densities across the six rootstock seedlings averaged 289 females/g fresh roots (range 87-431), with maximum and minimum infection on Rangpur lime and rough lemon, respectively (Table 5-10). The rootstock was the only factor contributing to the TTV in mean nematode female and juvenile root density counts.

Salinized potting mix had mean pH 6.8 and Ec 11.393 dS/m (data not shown). The Ec converted to an osmotic potential (π) of -4.101 MPa and total dissolved salts (TDS) of 7,292 ppm (3). The control potting mix had mean pH 7.1 and mean Ec 0.394 dS/m (π -0.142 MPa, TDS 252 ppm).

Discussion

Tylenchulus semipenetrans parasitism of roots consistently increased leaf Cl and Na in citrus seedlings with a wide range of salt tolerance under low salinity and neutral to slightly acidic conditions. *Tylenchulus semipenetrans* increased Na in leaves of sweet orange seedlings growing under high pH and high soil K, while the influence of the nematode on Cl in leaves had not been reported (Van Gundy and Martin, 1961). The effects of nematode on Cl accumulation in leaves were observed as early as 2 weeks after initiating low salt treatments (Appendix 2), suggesting that even shorter periods of salinity may be critical under high nematode densities. Because salt tolerance in citrus is defined as the ability of roots to exclude excess Cl and (or) Na from the shoots (Maas, 1993); thus high population densities of this parasite decrease salt tolerance in citrus rootstock seedlings.

The magnitudes of foliar Na accumulation due to *T. semipenetrans* infection under both saline (42-417%) and nonsaline (24-145%) conditions were comparable to those in Van Gundy and Martin's (1961) study at 14% soil Na (136%). However, the magnitude of Na accumulation due to parasitism at moderately high soil Na (9%) was higher (600%) than those in our study. The nematode induced reduction of K below a deficient range (Chapman, 1968) in this study confirmed

other greenhouse (Van Gundy and Martin, 1961) and field (Fouche et al., 1979; Milne and Willers, 1977) studies. Despite the high inoculum levels in these trials, the data did not confirm results from greenhouse studies where this parasite consistently decreased leaf Mn and Zn (Labanauskas et al., 1965; Van Gundy and Martin, 1961). Generally, the deficiencies of either Cu, Mn, or Zn in leaves induce the die-back of young twigs (Chapman, 1968), which is one of the symptoms of slow decline (O'Bannon and Esser, 1984; Tarjan and O'Bannon, 1987). Also, K deficiency results in smaller fruit and leaves (Chapman, 1968), while Cl and Na toxicities results in leaf chlorosis, leaf abscission, and stunted trees (Bohn et al., 1985; Chapman, 1968; Syvertsen et al., 1989; Waisel, 1972). This parasite could therefore, indirectly debilitate plant growth by increasing leaf Cl and Na to physiologically toxic levels or by reducing K to the deficient range (Chapman, 1968).

The reduced Cl and Na in nematode-infected roots confirmed the trends observed previously (Labanauskas et al., 1965). The reductions of Cl and Na in roots infected with this parasite and the subsequent accumulation of the two ions in leaves suggested a redistribution of these ions from roots to leaves. The reduction of K in both roots and leaves complicated any inference that we could make to clarify the redistribution of Cl and Na. Tarjan and O'Bannon (198) proposed that T. semipenetrans parasitism

alters the permeable nature of root cells, thus allowing trees to imbibe greater concentrations of some elements more and less of others. When high concentrations of salts occur in the soil, particularly when trees are irrigated with water of high salt content, leaf Cl and (or) Na levels detrimental to the tree results. However, that view (Tarjan and O'Bannon, 1984) explains neither the reduced root K, Cl, and Na nor the increased leaf Cl that we observed, and an alternative hypothesis is proposed.

Tylenchulus semipenetrans treatment increased the concentrations of starch in roots but did not affect any carbohydrate measured in the shoots. Thus, results of this study did not support the view that T. semipenetrans depletes carbohydrate reserves in citrus shoots (Hamid et al., 1985). Inasmuch as ion uptake requires metabolic energy (Marschner, 1986), the high concentration of carbohydrates in nematode-infected roots would suggest an increased uptake and the subsequent accumulation of nutrient ions in leaves. The accumulations of Cl and Na in leaves of infected plants supported this view; whereas foliar K deficiencies negated this hypothesis.

Photosynthates in plants are transported to storage organs as sucrose, which is osmotically active (Waisel, 1972). Chloride, Na, and K, which consistently responded to T. semipenetrans infection, are each also osmotically active in plant cells (Marschner, 1986; Waisel, 1972). The

increase in root carbohydrates associated with nematode infection was consistently accompanied by reductions in the concentrations of these ions. Therefore, high levels of assimilates in roots of nematode infected plants may reduce osmotic potential sufficiently to exclude osmotically active ions as a measure to counteract this reduction. Whereas Cl and Na accumulate in leaves, the increased Na in leaves could result in displacement of K. This hypothesis clarifies the reduced root Cl, K, and Na and the subsequent accumulation of Cl and Na in leaves, but it does not clarify the reduced foliar K. Further work is necessary, however, before it can be ascertained that increasing organic solutes as a factor in the displacement of the three ions in root cells.

Salinity accentuated the effects of T. semipenetrans on inorganic and organic solutes in all rootstocks. Also, salinity alone reduced K in both root and leaf tissues, along with Na in the roots. The effects of salinity on both leaf and root K confirmed other observations (Behboudin et al., 1986; Nieves et al., 1991). The interactions of salinity and T. semipenetrans are of practical concern because NaCl salinity in irrigation water is increasing (Bielorai et al., 1988; Chapman, 1968; Nabors, 1984; Syvertsen et al., 1989; Waisel, 1972); whereas there are no horticulturally acceptable citrus rootstocks that are both salt-tolerant and resistant to T. semipenetrans (Castle et

al., 1989). Salinity in most citrus-producing regions is cyclic (Bielorai et al., 1988; Syvertsen et al., 1989); however, cyclic salinity recently (Chapter 3) increased population densities of T. semipenetrans. Also, cyclic salinity recently (Chapter 4) reduced resistance to this nematode in commercially used nematode-resistant rootstocks. These studies together, demonstrated that the management of this parasite is even more critical in areas with salinity. Thus, resistance to T. semipenetrans should be genetically incorporated into salt-tolerant rootstocks or vice versa, to reduce population levels of this parasite while enhancing salt tolerance. This breeding approach has been successful in introducing resistance to certain pathogens in cereal cultivars with high tolerance to Na (Nabors, 1984).

CHAPTER 6
TYLENCHULUS SEMIPENETRANS INCREASES FOLIAR CHLORIDE AND SODIUM, BUT DECREASES NUTRIENT IONS IN CITRUS REPLANTS AND MATURE TREES

Introduction

Tylenchulus semipenetrans parasitism of citrus roots increased chloride (Cl) and sodium (Na) in leaves relative to uninfected citrus seedlings with a wide range of salt tolerance (Chapter 5). Salt tolerance in citrus is defined as the ability of roots to exclude excess Cl and (or) Na from shoots (Castle et al., 1989; Maas, 1993). Thus, when citrus roots were challenged by T. semipenetrans, salt tolerance was reduced. The nematode also reduced K and Cu in leaves, along with Cl, Na, and K in roots; whereas it increased starch in roots.

Van Gundy and Martin (1961) found that T. semipenetrans infection of citrus seedlings growing in soils with moderate to high exchangeable Na and K decreased Cu, Zn, and K in seedling leaves, but increased foliar Na. Labanauskas et al. (1965) confirmed that T. semipenetrans infection reduces foliar K in 3-year-old 'Valencia' oranges on sour orange rootstock. Also, the parasite reduced Ca, Mg, and Fe in leaves, and Cl, Na, K, B, and Fe in roots, and increased foliar P and B and root N, P, and Cu. Others (Fouche et al.

1977, Milne and Willers, 1979) verified that *T. semipenetrans* can also reduce K and Cu in leaves of mature citrus trees.

Tylenchulus semipenetrans induces slow decline (Cobb, 1914; Thomas, 1913) and replant (Martin and Bitters, 1961) disorders of citrus. Slow decline does not result in tree mortality, but replants with high *T. semipenetrans* infection rates may die within the first year (Thorne, 1961). Overall, symptoms of either disorder include stunting, die-back of young twigs, reduced yield, smaller leaves, leaf chlorosis, and leaf abscission (O'Bannon and Esser, 1985). These symptoms are similar to those induced by extreme ion toxicities and (or) nutrient ion deficiencies (Cohn et al., 1965; Cooper et al., 1962; O'Bannon and Esser, 1985). In fact, slow decline (O'Bannon and Esser, 1985) and replant (Bredell and Conradie, 1975) disorders are severe under salinity. The chemical composition of young citrus trees growing in old citrus soil and Cl and (or) Na composition of mature citrus trees infested with and without *T. semipenetrans* have not been studied. The objectives of this research were to compare the concentrations of nutrient elements and specific ion toxicities of citrus replants and mature trees each infested with high and low densities of *T. semipenetrans*.

Materials and Methods

Replants. The study was conducted in Ona, south central Florida, in an orchard with deep and well-drained soils. Old 'Parson Brown' orange (Citrus sinensis [L.] Osbeck) trees on sour orange (C. aurantium L.) rootstocks highly infected with T. semipenetrans, were removed February 1988, and the land prepared for replanting. Thirty, 37.5-m x 4.6-m plots were marked, and one-half fumigated with methyl bromide at the rate of 53 kg/ha. The other one-half were untreated control plots which remained infested with T. semipenetrans. The plots were blocked for both slope and soil color downslope. Young "Valencia" orange trees grafted on sour orange rootstocks were replanted in August 1988, at a density of five trees per plot. The young trees were fertilized with granular fertilizers three times per year (February, June, September) using fertilizer mixture and rates recommended for young trees under Florida conditions (Koo et al., 1984). Trees were also fertigated biweekly from March to September using urea (Koo et al., 1984). Pest management consisted of miticide application in spring and fall, and oil in summer. Trees were irrigated using microjets when necessary.

The first plant samples were collected in late spring 1992, before summer rainfall. Five mature leaves, which emerged during the 1991 spring were sampled from four

cardinal positions of each tree. The second leaf samples were collected in late summer 1992 from the 1992 early summer flushing twigs. These twigs had flat angular stems while those with fall leaves had ca. round stems. A shovel was used to collect soil and roots (20-25 cm depth) from four cardinal quadrants within the drip area of each plant when the first leaf samples were collected.

In the laboratory, leaf samples were washed with detergent and rinsed in tap water; whereas root samples were only rinsed. Each sample was pressed between tissue papers to remove excess water. Samples were dried at 70 C for 48 hours, then ground in a Wiley mill to pass a 375-μm-pore sieve. The concentrations of Na, Ca, K, Mg, P, Cu, Fe, Mn, and Zn in leaves and roots were analyzed (Rhue and Kidder, 1983) using an inductively coupled plasma emission spectrometer (Perkin Elmer Co., Norwalk, CT). The concentrations of Cl in root and leaves were analyzed using a Haake Chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ); whereas those of starch were analyzed using the method of Smith (1980).

Nematodes were extracted during a 48-hour period from a subsample of 50 cm^3 soil using Baermann tray method (Baermann, 1917) and counted. Soil reaction (pH) and electrical conductivity (Ec_e) were measured from the saturation extract of previously air-dried soil samples. The saturation extract was prepared by mixing soil and water

in a 1:2 ratio (v/v), which was then stirred for 30 seconds and incubated for 30 minutes. Soil texture (Bouyoucos, 1927) and organic matter (Rhue and Kidder, 1983) were measured from the remaining soil samples.

Data were first evaluated for a block effect using analysis of variance (ANOVA), but this factor was not significant ($\underline{P} \leq 0.10$). Subplot data were pooled by plot, and analyzed using t-test. Because nematode densities were widely variable, the relationships between Cl, Na, and K and starch with nematode densities were evaluated using linear regression. Prior to analysis, ion and nematode data were transformed into ln(x) and ln(x+1), respectively.

Mature citrus trees. The study was initiated during autumn 1991 in a mature orchard with ca. (25-year-old) "Valencia" orange trees on sour orange rootstocks in Vero Beach, eastern coast of Florida. This citrus-producing area is known for its shallow-poorly-drained soils, with salinity problems (O'Bannon and Esser, 1985). Trees were planted on irrigation beds, and irrigated with furrow irrigation systems when necessary. Fertilization and pest management were as recommended for mature tree under Florida conditions (Koo et al., 1984). The purpose of the initial survey was to identify trees with and without T. semipenetrans, and the sample was collected from 20 randomly selected trees. The soil was turned to ca. 10-25 cm depth, and a handful of soil taken from eight positions under the canopy and the samples

combined. Nematode juveniles were extracted and counted as described for the replants.

After trees with and without nematodes were identified, the study was continued in 11 rows starting from the southwestern corner of the grove. The samples were collected in late summer (1992) from trees with and without nematodes, and from trees with unknown nematode status, resulting in a total of 60 trees. Sixteen soil cores were collected at random under the canopy, and combined.

Nematodes were extracted from soil as described for the replants. The concentrations of Cl, Na, and K in leaves, and starch in leaves and roots, soil reaction, Ec_e, soil moisture, and soil texture were analyzed as described for replants. The data were grouped into high (> 900 juveniles/100 cm^3) and low (< 9 juveniles/100 cm^3 soil) nematode-infested trees, and analyzed as described for replants. Data where t-test was significant ($P < 0.05$) are discussed, unless stated otherwise.

Results

Replants. *Tylenchulus semipenetrans* population densities in methyl bromide (MB) treated plots averaged 27 juveniles/100 cm^3 soil (range 0-263); whereas those in

TABLE 6-1. Soil characteristics of citrus replant plots in south central Florida and of an orchard with mature trees in the eastern coast of Florida with trees infested with low and high densities of *Tylenchulus semipenetrans*.

Soil variables		Nematode infestation levels in: Replants Low	Replants High	Mature trees Low	Mature trees High
Texture	sand	95.7	96.4 ns	94.8	95.6 ns
	silt	1.9	1.8 ns	3.0	1.4 *
	clay	2.4	2.8 ns	2.2	3.0 ns
Ec (dS/m)		3.3	3.5 ns	4.8	4.9 ns
pH		6.8	6.6 ns	6.2	6.3 ns
Organic matter		2.1	2.2 ns	-	-

Each value for replants is an average of 15 replicates; whereas values for mature trees are averages of 16 and 18 replicates for low and high infestations, respectively.

* Significant at $P \leq 0.05$; ns = not significant at $P \leq 0.10$.

untreated plots were much higher, averaging 2,476 juveniles/100 cm^3 soil (range 170-6,670). The mean Ec_e, pH, and organic matter in treated and untreated plots were not different (Table 6-1).

Replants in untreated control plots had lower K (48%), Mn (16%), Zn (15%), and Cu (14%), but higher Cl (80%), Na (36%), and Mg (9%) in fall leaves (dry season) than those in

TABLE 6-2. Foliar concentrations of four macronutrients (% dry weight) and three micronutrients (ppm dry weight) in citrus replants with low and high densities of _Tylenchulus semipenetrans_ (per 100 cm^3 soil).

			Nematode infestation	
Season	Tissue	Variable	Low	High
Rainy		Cl (ppm)	412.00	467.07 **
Dry	Leaf	Cl	0.10	0.18 **
		K	1.34	0.70 **
		Mg	0.33	0.36 †
		Na	0.14	0.19 *
		Cu	20.00	17.00 *
		Mn	20.00	17.13 *
		Zn	25.36	20.89 *
		Starch	2.34	3.18 *
Dry	Root	Cl	0.47	0.42 †
		K	1.40	1.28 ns
		Na	0.21	0.12 **
		Starch	3.80	3.90 ns

Each value is mean of 15 replicates.

Rainy season" = sample of foliage produced in spring 1992 and collected in late summer 1992.

"Dry season" = sample of foliage produced in fall 1991 and collected in late spring 1992.

** Significant $\underline{P} \leq 0.01$, * $\underline{P} \leq 0.05$, † $\underline{P} \leq 0.10$; ns= not significant at $\underline{P} \leq 0.10$.

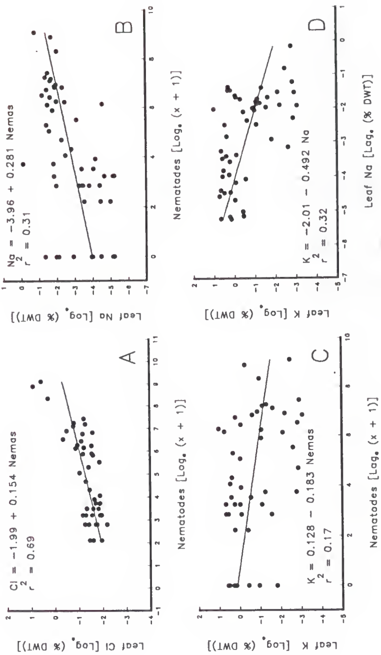
TABLE 6-3. Concentrations (% dry weight) of leaf osmoticum ions in mature citrus trees with low and high densities of *Tylenchulus semipenetrans* (per 100 cm^3).

		Nematode infestation	
Tissue	Variable	Low	High
Leaf	Cl	0.19	0.85 **
	K	1.03	0.27 **
	Na	0.14	0.23 **

Each mean under Low and High is an average of 12 and 10 figures, respectively.
**Significant $P \leq 0.01$; ns=not significant at $P \leq 0.10$.

MB plots (Table 6-1). Summer leaves from untreated plots had 14% more Cl than those in MB plots. The treatments did not affect Ca, Fe, and P (data not shown). Replants on untreated plots also had lower Cl (11%) and Na (43%) in fall roots, with K showing a declining trend. Treatment effects on other ions in roots were not different (data not shown). Relative to MB plots, plants in untreated plots had high starch in both roots (3%, $P \geq 0.10$) and leaves (36%). The concentrations of fall foliar Cl ($r = 0.63$, $P \leq 0.01$) were positively correlated with densities of *T. semipenetrans*, those of foliar K ($r = -0.66$, $P \leq 0.01$) were negatively

FIGURE 6-1. Ion-*Tylenchulus semipenetrans* and ion-ion relationships in mature citrus trees in the east coast of Florida: A) Leaf chloride versus nematode densities, B) Leaf sodium versus nematode densities, C) Leaf potassium versus nematode densities, D) Leaf potassium versus leaf sodium.



A
Cl = −1.99 + 0.154 Nemas
r^2 = 0.69
Leaf Cl [Log$_e$ (% DWT)]
Nematodes [Log$_e$ (x + 1)]
B
Na = −3.96 + 0.281 Nemas
r^2 = 0.31
Leaf Na [Log$_e$ (% DWT)]
Nematodes [Log$_e$ (x + 1)]
C
K = 0.128 − 0.183 Nemas
r^2 = 0.17
Leaf K [Log$_e$ (% DWT)]
Nematodes [Log$_e$ (x + 1)]
D
K = −2.01 − 0.492 Na
r^2 = 0.32
Leaf K [Log$_e$ (% DWT)]
Leaf Na [Log$_e$ (% DWT)]

correlated with nematode levels; whereas neither Na nor root starch was correlated with nematode densities. Leaf K was negatively correlated with leaf Na ($r = -0.63$, $\underline{P} \leq 0.01$).

Mature trees. Soil variables between the light and heavy infested trees were not different, except for higher silt in poorly infested plots (Table 6-1).

Nematode densities in highly infested trees averaged 3,785 juveniles/100 cm^3 soil (range 933-9,323); whereas those in poorly infested trees averaged 1 juvenile/100 cm^3 soil (range 0-9). Nematode densities were correlated with neither pH, soil moisture, soil texture, nor Ec, suggesting that the patchy distribution of nematodes in this grove was random.

Relative to poorly infected trees, (Table 6-2), highly infected trees had higher foliar Cl (34%) and Na (64%), but lower K (74%). The concentrations of starch in both roots and leaves for the two treatments were not different. Foliar Cl ($r = 0.83$, $\underline{P} \leq 0.01$) and Na ($r = 0.56$, $\underline{P} \leq 0.01$) each were positively correlated with nematode densities; whereas foliar K was negatively correlated with nematode levels ($r = -0.41$, $P < 0.01$; Figure 6-1). Leaf K was negatively correlated with leaf Na ($r = -0.57$).

Discussion

Nematode densities were not correlated with other soil variables, suggesting that the observed distribution was random. All other soil variables between poorly and heavily infested plots in both mature and replant trees were not different, except for silt in mature trees. The higher concentration of silt was in poorly infested plots, suggesting that this variable was not responsible for the patchy distribution of nematodes in the orchard with mature trees.

Tylenchulus semipenetrans affected the concentrations of nutrient elements and toxicity ions as in the greenhouse studies (Chapter 5; Van Gundy and Martin, 1961) and other field studies (Fouche et al., 1977; Milne and Willers, 1979).

Foliar K and Mn in trees in MB plots were, respectively, optimum and low (Chapman, 1968; Koo et al., 1984), but both ions were deficient in trees growing on untreated plots. Foliar Zn and P in both treatments were low, whereas Ca, Mg, and Fe were optimum. Foliar Cl and Na on untreated plots were high, while those on treated plots were low. In mature trees, both trees with and without nematodes had K deficiencies in leaves. Relative to the deficiency threshold level for leaf K (1.7%; Koo et al., 1984), the deficiency was 84% more severe in trees with a

heavy nematode load; whereas in with a light nematode load the severity was 39%. Average foliar Cl and Na in replants heavily infested with nematodes was within the physiologically toxic range (Chapman, 1968). In mature trees, both Cl and Na in trees with high nematode densities were above the physiologically toxic level; whereas they were below this level in trees with low nematode counts.

Tylenchulus semipenetrans parasitism of roots increased foliar Cl 80% above those of MB plots during the dry season and by 14% during the rainy season. The 1991 fall flushing leaves were on the trees during the dry season and thus, periodically received water from supplemental irrigation, which is inherently saltier than rain water. This, together with the longer duration that the leaves were on the trees prior to sampling compared with summer leaves, could explain the higher accumulation of Cl in fall than in summer leaves. Although accumulation of Cl in citrus leaves due to the citrus nematode may be independent of the season, the data suggest that this may be critical during dry seasons, particularly when supplemental irrigation water is of poor quality.

Nematode-infected replants had high starch in both roots and leaves. In a salt tolerance study (Chapter 5) it was shown that T. semipenetrans parasitism of roots increases root starch. These data do not support the view

that this parasite depletes carbohydrates in citrus plants (Hamid et al., 1985).

The constant terms in the linear regression models of ions versus nematode densities were small. For instance, rearranging K in the model for mature trees, 7,169 juveniles per 100 cm^3 soil are required to reduce K to the deficiency range of 1.7%; whereas 3,711 juveniles per 100 cm^3 soil would increase Cl to the 1% physiological toxic threshold level. Thus, high population densities of _T_. _semipenetrans_ in soil are necessary to induce ionic imbalances in citrus. High population densities of _T_. _semipenetrans_ with comparable magnitude in densities occur in most citrus orchards. For instance, the average nematode densities in replants (2,467 nematodes/100 cm^3 soil) and in mature trees (3,785 nematodes/100 cm^3 soil) were comparable to mean nematode densities in California (Hamid et al., 1985), Florida (Duncan and Noling, 1990), Israel (Cohn et al., 1965), Texas (Heald, 1977), and South Africa (Cohn, 1976; Milne and Willers, 1979) under field conditions. It was previously established that when the citrus nematode density of was < 2,000 juveniles/100 cm^3 soil in Florida (Duncan and Noling, 1980), < 4,000 juveniles/g fresh roots in Israel or South Africa (Cohn, 1976), and < 700 females/g fresh roots in California (Hamid et al., 1985), citrus trees did not respond to nematicidal treatments (Duncan and Cohn, 1990). Similarly, the high inoculum levels (196,000

juveniles/plant) that induced ionic imbalances in salt tolerant study (Chapter 5), explain why inoculations with 20,000 juveniles/plant (Labanauskas et al., 1965; Van Gundy and Martin, 1961) did not result in significant ionic interactions.

The gradual accumulation of excess Cl and (or) Na in leaves, concomitant with nutrient ion imbalances in citrus infected by T. semipenetrans, define symptoms of slow decline and replant disorders of citrus. For instance, slow decline is associated with die-back of young twigs; whereas foliar Cu and Fe deficiencies are known to induce this symptom (Chapman, 1968). Similarly, smaller leaves and fruit are generally related to K deficiencies; whereas leaf chlorosis and leaf abscission are related to Cl and (or) Na toxicity (Cooper et al., 1962).

Salinity aggravated the effects of T. semipenetrans on nutrient imbalances and accumulation of toxic ions in leaves. Others noted that slow decline (O'Bannon and Esser, 1985) and replant (Bredell and Conradie, 1976; Burger and Bruwer, 1979) disorders of citrus are severe in areas with salinity. Thus, nutrient imbalances and accumulation of Cl and (or) Na to physiologically toxic levels in leaves may be mechanisms by which T. semipenetrans induces slow decline and replant disorders of citrus.

CHAPTER 7
SALINITY INCREASES TYLENCHULUS SEMIPENETRANS DENSITIES THROUGH SYSTEMIC EFFECTS, BUT THE NEMATODE INCREASES CHLORIDE AND SODIUM IN CITRUS LEAVES THROUGH NONSYSTEMIC EFFECTS

Introduction

High densities of the nematode *Tylenchulus semipenetrans* Cobb may occur in citrus-producing regions with salinity (Cohn, 1976; Cohn et al., 1965; Machmer, 1958). However, osmotic potential of -1.01 MPa reduced *T. semipenetrans* juvenile motility; whereas -4.05 MPa completely restricted motility (Viglierchio et al., 1969). In fallow soil, salinity reduced egg-hatch and infectivity of this nematode, but when salinity was removed both activities resumed (Dropkin et al., 1958; Kirkpatrick and Van Gundy, 1966).

Recently (Chapters 3,4), it was demonstrated that cyclic salinity can reduce resistance to *T. semipenetrans* in citrus rootstock seedlings representing a wide range of resistance to this nematode. Also, *T. semipenetrans* parasitism of roots reduced salt tolerance in citrus rootstock seedlings representing a wide range of salt tolerant germplasm (Chapter 5). The mechanisms by which *T.*

semipenetrans and salinity interacts in citrus are not known, but likely involve water relations, mineral nutrition, and growth.

Seedlings with split-root system may facilitate the characterization of the reciprocal interactions between *T. semipenetrans* and salinity interactions, because specific treatments can be compartmentalized on portions of the root. Whether the reciprocal interactions salinity and nematodes are translocatable through the plant (i.e. systemic), has not been studied. Thus, this research was initiated: 1) to measure whether the effects of salinity on the population densities of *T. semipenetrans* may be translocated from the salinized nematode-free to the nonsalinized nematode-infected root half, 2) to test whether the effects of *T. semipenetrans* infection can be translocated from the infected nonsalinized to the salinized root half, thereby enhancing accumulation of Cl and (or) Na in leaves, 3) to compare the allocation of photosynthates in *T. semipenetrans*-infected and noninfected half-root systems within the same plant, and 4) to compare the interactions of salinity and *T. semipenetrans* stresses when applied together in one-half, or separately in either half of the same plant.

Materials and Methods

Taproots of 6-month-old sour orange (*Citrus aurantium* L.) rootstock seedlings were vertically split into two halves and the joint at ca. 5 cm height above the soil surface mark secured with Parafilm M (American Co., Greenwich, CT). Each seedling was transplanted into two 15-cm-diam clay pots containing soil mix of 3:1 (v/v) steamed autoclaved sand (97% sand, 2% silt, 1% clay; pH 7.1; 2% organic matter) and PRO-MIX B (Premier Brands, Inc., Stamford, Canada). Seedlings were allowed to develop and establish root halves during a 6-week period.

Seedlings were initially irrigated with 100 ml tap water/pot every other day, and then with 250 ml of water after the first shoot flush. Plants were fertilized weekly with a solution of 2.5 g of 20:20:20 ($N:P_2O_5:K_2O$) mixture per liter water at the same rate as irrigation water, and biweekly with a 25% Hoagland's solution (Hoagland and Arnon, 1950) to provide micronutrients. Ambient greenhouse temperatures averaged 28 C maximum (range 26-31 C) and 25 C minimum (range 23-27 C).

Seedlings were selected for uniformity 6 weeks after transplanting. The experimental half-root treatments include: Untreated/Untreated (0/0), Tylenchulus/0 (T/0), Salinity/0 (S/0), S/S, T/T, T/S, TS/0, and TS/TS, each

treatment with 10 replicate plants. Seedlings were arranged on a greenhouse bench in a randomized complete block design.

Nematodes for inoculum were collected, extracted, and disinfested as described previously (Chapter 3). However, nematodes were further separated from soil particles and debris using the centrifugal-flotation method (Jenkins, 1964). The inoculum was poured into two nested 25-μm-pore sieves, rinsed on the sieves with tap water for 5 minutes, washed in 500 ml of 0.10% $CuSO_4$ solution, and aerated for 30 minutes. The inoculum was again poured into the two nested sieves, which were previously soaked in the disinfectant solution for 30 minutes, and rinsed for 5 minutes using tap water. The filtrate was mixed in water agar and plated on PARP medium for Phytophthora spp. (Timmer et al., 1988), which after a 24-hour incubation tested negative. The nematode treatment was initiated 6 weeks after transplanting by each pot with ca. 350,000 juveniles four times over a 3-day interval. The remaining uninoculated treatments were inoculated with nematode inoculum filtrate (25-μm-pore sieve) to establish microbial consistency other than nematodes in the treatments. Salinity was initiated 4 weeks after inoculation by irrigating the salt treated halves with 25 mols $NaCl/m^3$ H_2O + 3.3 mols $CaCl_2/m^3$ H_2O for 12 weeks.

At harvest, 22 weeks after transplanting, nematodes were extracted from 1 g fresh roots/plant, stained, and counted as described previously (Chapter 3). The remaining

roots and shoots were dried at 70 C for 48 hours, weighed, and then roots and leaves separately ground in a Wiley mill to pass a 375-μm-pore sieve. Potassium and Na from 1 g leaf tissues and Na, and K from root tissues were analyzed (Rhue and Kidder, 1983) by an inductively coupled plasma emission spectrometer (Perkin Elmer Co., Norwalk, CT); whereas Cl from 1 g of root and leaf tissues each was using a Haake Chloridometer (Haake and Buchler Instruments, Inc., Saddle Brook, NJ). Root starch was analyzed using methods of Smith (1981, see Chapter 5) in root halves of T/0 treatment.

Data were analyzed using only those single degrees of freedom orthogonal contrasts appropriate to each objective. (1) To measure whether salinity effects on T. semipenetrans (Chapter 3,4) are systemic, nematode levels in the infected root half of the T/S and T/0 treatments were compared. When nematode densities in T/S > T/0, a systemic effect of salinity on nematode densities was operative; whereas when nematode densities in T/S $\leq$ T/0 would suggest a nonsystemic effect. Also, nematode levels in TS/0 and T/0 were compared to evaluate the direct effects of salinity on nematode densities. Because the sample variances were heterogenous, nematode data were transformed using $\ln(x+1)$ prior to analysis, but untransformed data are discussed. (2) To test whether the effects of T. semipenetrans on the accumulation of Cl and (or) Na in leaves were systemic, foliar Cl and Na of the T/S and S/0 treatments were

compared. When the concentration of the given ion in T/S > S/0, a systemic effect due to nematodes was operative for that ion; whereas if the ion in T/S $\leq$ S/0, a nonsystemic effect was suggested. Also, foliar Cl and Na in T/S and TS/0 treatments were contrasted to evaluate the direct effects of nematodes on salt ions. (3) To compare the effects of T. semipenetrans infection on the allocation of nonstructural carbohydrates in roots, root starch in nematode-treated and untreated root halves of the T/0 treatment were compared.

(4) To compare damage potential of t. semipenetrans and salinity when compartmentalized and separated, plant growth measurements in T/0, S/0, T/s, and TS/0 treatments were compared.

Results

Nematode densities. Relative to T/0, female, juvenile, and egg counts in T/S were higher by 77%, 105%, and 80%, respectively (Table 7-1). The T/S treatment also increased females (33%), juveniles (84%), and eggs (66%) above the TS/0 treatment. Relative to other treatments, the TS/TS suppressed nematode population densities most (Appendix 15).

TABLE 7-1. Tylenchulus semipenetrans (T) female, juvenile, and egg counts per gram of fresh roots of sour orange seedlings with split-roots treated with (S) and without (0) low salinity.

	Nematodes/g fresh roots		
Treatment	Females	Juveniles	Eggs
T/0	564	1,768	17,295
T/S	999	3,617	31,157
TS/0	724	1,968	18,805
Contrasts:			
T/0 vs. T/S	***	***	***
T/0 vs. TS/0	ns	ns	ns
T/S vs. TS/0	***	***	***

** Significant $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

Foliar osmoticum ions. Relative to S/0, the T/S treatment did not affect foliar Cl or Na (Table 7-2). Foliar Cl in T/S were lower (43%) than those in TS/0; whereas foliar Na in both treatments did not differ. Overall, K was reduced much more in the T/S than in the TS/0

TABLE 7-2. Spatial effects of Tylenchulus semipenetrans (T) with (S) and without (0) low salinity on foliar osmoticum ions (% dry weight) of sour orange seedlings with split-roots.

Treatments	Cl	Na	K
S/0	0.61	0.43	1.54
T/S	0.52	0.39	1.42
TS/0	0.92	0.34	1.81
Contrasts			
S/0 vs. T/S	ns	ns	ns
S/0 vs. TS/0	ns	ns	ns
T/S vs. TS/0	**	ns	**

** Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

TABLE 7-3. The partitioning of the concentrations (%) of starch , chloride (Cl), sodium (Na), and potassium (K) in two root halves as affected by Tylenchulus semipenetrans infecting half-root system of sour orange seedlings with split-roots.

Half-root	starch (%)	Cl	Na	K
Untreated (0)	2.94b	0.40a	0.51a	2.21a
Nematode (T)	5.14a	0.31b	0.28b	1.09b

Column means (n = 10) with the same letter are not different ($P \leq 0.05$) according to t-test.

TABLE 7-4. Effects of *Tylenchulus semipenetrans* (T) and salinity (S) separated or combined on dry shoot and root weights and shoot height of sour orange with split-roots.

	Weight (g)		Height
Treatment	Shoot	Root	(cm)
S/0	10.28	3.49	25.4
T/0	11.72	3.40	26.5
T/S	10.41	2.94	25.6
TS/0	8.52	2.86	22.6
Contrasts:			
S/0 vs. T/0	ns	ns	ns
S/0 vs. T/S	ns	ns	ns
S/0 vs. TS/0	ns	ns	ns
T/0 vs. T/S	ns	ns	ns
T/0 vs. TS/0	**	**	ns
T/S vs. TS/0	**	ns	ns

** Significant $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

treatment. The TS/TS treatment had the most impact on the three ions than any other treatment (Appendix 16).

Allocation of starch. The plant stored much more starch in the half-root infected with nematodes compared with the untreated half (Table 7-3). Relative to the

uninfected half, the infected half had 75% more starch. However, the infected root-half had lower Cl (23%), Na (45%), and K (51%) than the uninfected half.

Growth characteristics. There were no treatment effects on growth variables when one-half root system was untreated, regardless of the kind or number of treatments on the other half-root system (Table 7-4). However, when salinity and nematodes together occupied both root-halves (TS/TS), growth reduction was severely affected (Appendix 17).

Discussion

The high *T. semipenetrans* densities when salinity stress on nematodes was indirect confirmed recent studies (Chapters 3,4), where cyclic salinity increased population levels of this nematode. High field densities of this parasite occur in citrus-producing regions with salinity (Cohn, 1976; Cohn et al., 1965; Machmer, 1958). Thus, this and recent findings where leaching soluble salts from the root zone increased population densities of *T. semipenetrans* (Chapters 3,4), substantiate the conditions which enhance population densities of this nematode in orchards with salinity.

The high population densities in T/S relative to TS/0 treatment, illustrated the systemic effect of salinity on

nematode female, juvenile, and egg root densities. High T. semipenetrans densities and high concentrations of salinity in citrus groves tend to be spatially separated, with nematodes in upper and salinity in lower soil horizons (Bohn et al., 1985, Inserra et al., 1975). Thus, this study demonstrated that nematode population densities would be increased under salinized conditions, even in roots spatially separated from the most direct source of salinity. Relative to nematode densities in T/T, direct salinity in TS/TS resulted in declining trends of root count densities for all stages (Appendix 15). Because in TS/0 similar trends were not observed, it may be that in addition to direct salinity stress on nematodes, the TS/TS treatment suppressed population densities through providing food of less quality when compared to the TS/0 treatment.

Comparison of S/0 with TS/0 and T/S treatments, the T/S treatment illustrated the nonsystemic effects of infection of roots by T. semipenetrans on Cl and Na accumulation in citrus leaves. The TS/0 treatment increased foliar Cl almost twice as much as T/S; whereas the Cl in the latter treatment was not different than in S/0.

Although the data in this study did not demonstrate systemic effects of T. semipenetrans infection on Cl and Na, the root data supported the hypothesis that nonstructural carbohydrates may be involved in the alteration of the partitioning of osmotically active ions in citrus. Starch

in root halves infected with nematodes increased 75% above the noninfected halves in T/0. However, when expressed as content (mg/dry weight), the starch weight were not different. This suggests that T. semipenetrans did not create an apparent sink for photosynthates, but starch accumulated because of the inefficiency of the infected root to incorporate nonstructural carbon into structural carbon. Similar inefficiencies were observed in half-roots inoculated with mycorrhiza (Dixon et al., 1988; Koch and Johnson, 1984).

Tylenchulus semipenetrans infection reduced Cl, Na, and K in infected root-half compared with the noninfected half in the same plant. The reduction of these ions with increasing concentrations of starch in the same root halves supported the hypothesis which proposes that increasing root carbohydrates displaces Cl, Na, and (or) K in roots (Chapter 5). Also, if this hypothesis is valid, the unequal distribution of starch in the root halves of the T/0 treatment supports the observed nonsystemic effects of this parasite on either Cl and Na in the T/S treatment.

Foliar Cl in TS/TS treatment (Appendix 16) was within the 1.35-2.77% range where clinical symptoms of Cl toxicity occur (Cooper et al., 1952). A few seedlings in this treatment had leaf chlorosis. The longer duration of salt treatment and the moderately high inoculum densities compared to the previous study, were meant to counteract

enhanced plant growth which was probably due to unlimited soil area for root growth. Reduced plant growth in TS/TS treatment (Appendix 17) might have decreased the dilution effect more, so that eventually the seedlings became more stressed from the toxicity effects and (or) osmotic stress of Cl and (or) Na.

Generally, this parasite decreases K in citrus leaves (Chapters 5,6; Fouche et al., 1977; Milne and Willers, 1979; Van Gundy and Martin, 1961). In contrast to Cl, the T/S treatment reduced foliar K more than TS/0, which slightly elevated K ($\underline{P} \leq 0.10$) above the 0/0 control. The increment in foliar K in TS/0 could have been due to increased absorption surface area in untreated half, as demonstrated by increased mean root weight (data not shown). The increased root growth in untreated halves also may clarify the lack of significant differences in foliar K in T/0 and S/0 treatments with the 0/0 control. Previously, Zekri (1988) demonstrated that growth of untreated halves could be enhanced during reduction of the stressed half, and that the untreated half-root could also sustain citrus seedling requirements for water and nutrient ions.

Shoot and total root weight and plant height when salinity and T. semipenetrans were spatially separated (T/S) or applied together (TS/0) were not different. Shoot weight was lowest when both nematodes and salinity occupied one or both root halves than when each factor was alone in one or

both root halves (T/T, S/S, T/0, S/0) or when both factors were spatially separated (T/S).

Under some soil conditions, ca. 95% of _T_. _semipenetrans_ population densities inhabit soil horizons in the first 1.80 m depth (8), where most of the roots occur under irrigation. Under moist conditions, salinity is more concentrated with soil depth (Bohn et al., 1985), so that salinity and nematodes may remain spatially separated. However, when high population densities of _T_. _semipenetrans_ are not managed, they may reduce fibrous roots in upper soil horizons to an extent that the plant responds by redistributing growth to root portions at greater soil depth. Because of the proximity to concentrated soil solutions in these depths, the increased absorption root surface may not be beneficial to plant growth when it serves as a vehicle for absorbing and transporting more Cl and Na to shoots.

Alternatively, fluctuating moist conditions in the first 1.80 m depth lead to cyclic salinity in this zone (Bohn et al., 1985), thus creating conditions that enhance both population densities (Chapters 3,4) and salinity damage (Chapter 5; TS/TS, TS/0). In contrast, limited soil moisture fluctuations spatially separate nematodes and salinity (as in T/S), resulting in less Cl accumulation in leaves than when compartmentalized. The observation that TS/0 or TS/TS increased Cl accumulation and decreased shoot

growth more than other treatments, supports the hypothesis that the effects of _T. semipenetrans_ parasitism on citrus roots are more severe in citrus-producing regions with salinity (Bredell and Conradie, 1975; O'Bannon and Esser, 1985). Also, this observation supports the hypothesis that ionic imbalances are integral components in _T. semipenetrans_ induced slow decline of citrus (Chapters 5,6).

CHAPTER 8
MECHANICAL ROOT PRUNING SIMULATES THE EFFECTS OF <u>TYLENCHULUS SEMIPENETRANS</u> ON OSMOTICUM IONS AND STARCH IN CITRUS

Introduction

The mechanism by which the citrus nematode, *Tylenchulus semipenetrans* Cobb, affects osmotically active ions (Cl,K,Na) in citrus is not known. This nematode reduces foliar K (Chapters 5,6,7; Fouche et al., 1977; Milne and Willers, 1979; Van Gundy and Martin, 1961), along with root Cl, Na, and (or) K (Chapters 5,6,7; Labanauskas et al., 1965), but increases the accumulation of excess Cl and (or) Na in leaves (Chapters 5,6,7; Van Gundy and Martin, 1961). Tarjan and O'Bannon (1987) proposed that the mechanism by which *T. semipenetrans* parasitism may alter the permeable nature of root cells, thus allowing trees to imbibe greater concentrations of some elements more and less of others.

Girdling stems of healthy citrus trees reduced nonstructural carbohydrates (CHO) in roots, and resulting in more Na being accumulated in roots than in roots of control trees (Rodney et al., 1956). Also, the collapse of lemon on sour orange rootstocks, associated with reduced CHO in roots, results in high Na in roots (Rodney et al., 1956). In contrast, *T. semipenetrans* parasitism reduces Cl and, Na,

and (or) K in roots; whereas such roots have high levels of CHO (Chapters 5,6,7). Similarly, challenged with salinity or mycorrhiza have higher levels of CHO in roots (Chapter 5; Dixon et al., 1988; Koch and Johnson, 1984; Walker et al., 1984; Williams et al., 1991) and lower concentrations of root Cl, Na, and (or) K (Behboudin et al., 1986; Chapter 5; Graham and Syvertsen, 1989; Hartmond et al., 1987). Thus, it appears that increasing CHO in roots reduces the concentrations of Cl, Na, and K in citrus roots. The effects of increasing root nonstructural carbohydrates on the partitioning of osmotically active ions in citrus have not been studied. The objectives of this research were to test whether inducing accumulation of nonstructural CHO in roots by mechanical root pruning would alter the partitioning of Cl, Na, and K as does parasitism of _T. semipenetrans_.

Materials and Methods

The initial study was conducted using Cleopatra mandarin (_Citrus reticulata_ Blanco) in a soil mix of 3:1 (v/v) steamed autoclaved sand (97% sand, 2% silt, 1% clay; pH 6.8, 2% organic matter) and PRO-MIX BX (Premier Brands, Inc., Stamford, Canada). Seventy 15-cm-diam clay pots were each partitioned in half by an aluminum wrapped polypropylene screen. The taproots of 5-month-old seedlings were vertically split in two and the joint 10 cm above the

soil surface firmly secured with Parafilm M (American Company, Greenwich, CT). Roots were trimmed so that each half-root system had ca. the same number of fibrous roots, and each seedling transplanted so that each of the two halves occupied half of the pot volume. The joint remained above the surface of the soil mix, and seedlings were allowed to develop and establish the root systems during an 8-week period.

Plants were irrigated with 250 ml tap water/plant every other day, and fertilized weekly at the same rate as irrigation water with a solution of 5 g of a 20:20:20 ($N:P_2O_5:K_2O$) mixture per liter of water. A 25% Hoagland solution (Hoagland and Arnon, 1950) was added once biweekly to provide micronutrients at the same rate as irrigation water. Ambient temperatures averaged 28 C maximum (26-30 C) and 25 C minimum (22-27 C).

The experimental treatments were nematode, pruning, untreated controls, each with 20 replicates. Sixty plants were selected for uniformity 2 months after transplanting and arranged in a randomized complete block design on a greenhouse bench. Nematode inoculum was collected, extracted from roots, and disinfected as described previously (Chapter 7). Each nematode treatment seedling was inoculated two times with a total of ca. 50,000 nematodes (25,000/half root system) at a 3-day interval (Chapter 3). Pruned and control plants were inoculated with

nematode filtrate (25-μm-pore-sieve) to establish in their rhizosphere any microbes associated with the inoculum.

The pruning treatment was initiated by excising one of the half-root systems ca. 1 cm below the 5-cm-high joint 7 weeks after inoculation. Excised half-root systems were lifted from one compartment with minimum disturbance to the remaining halves. The remaining stump was sealed and secured with Parafilm against the intact stem. Ten plants in pruned, nematode, and control treatments each were salinized starting 3 days after pruning using 30 mols $NaCl/m^3$ H_2O + 5.3 mols $CaCl_2/m^3$ H_2O in 250 ml solution/plant every other day. All plants were harvested 14 days after salinization.

Nematodes were extracted from 1 g of fresh roots/plant, separated from debris, stained, and counted as described previously (Chapter 3). Shoots and the remaining roots were dried at 70 C for 48 hours, weighed, and each ground in a Wiley mill to pass a 375-μm-pore sieve. Chloride in roots and leaves was analyzed (Rhue and Kidder, 1983) using Haake Chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ), and K and Na in leaves and roots were analyzed (Rhue and Kidder, 1983) by an inductively coupled plasma emission spectrometer (Perkin Elmer Co., Norwalk, CT). Starch, ketone sugars, and reducing sugars in roots were analyzed using standard analytical methods (Nelson, 1944; Roe et al., 1949; Smith, 1981; see Chapter 5).

The entire experiment was repeated once using 6-month-old sour orange (C. aurantium L.) seedlings. Methods, materials, and greenhouse conditions were as described for Cleopatra mandarin except that salt was added as 50 mols $NaCl/m^3$ H_2O + 8.8 mols $CaCl_2/m^3$ H_2O and root carbohydrates were not analyzed. Ion data were expressed as concentration (% dry weight) and as content (mg/organ weight), however, because treatment effects were independent of the unit used, only the concentration units are discussed. Treatment effects were analyzed using analysis of variance (ANOVA), and mean differences were separated using Duncan's multiple-range test. Nematode data were transformed into ln(x+1) prior to ANOVA to homogenize the variance (Little and Hills, 1975), but untransformed data are discussed. Only data where the F-test was significant ($P \leq 0.01$) are discussed unless indicated otherwise. The effects of root pruning and nematode treatments are each discussed relative to the untreated controls unless stated otherwise.

Results

Generally, pruning and T. semipenetrans each increased nonstructural CHO in Cleopatra mandarin roots except reducing sugars in nematode-infected roots (Table 8-1).

TABLE 8-1. Concentrations of root carbohydrate (% dry weight) of Cleopatra mandarin seedlings as affected by root pruning and Tylenchulus semipenetrans infection with and without low salinity.

Root	Starch		Ketone sugars		Reducing sugars	
treatment	No salt	Salt	No salt	Salt	No salt	Salt
Control	1.61c	2.07c	2.28c	2.26b	0.44b	0.50b
Nematode	2.23b	2.50b	2.46bc	2.32b	0.30b	0.35c
Pruned	3.75a	4.39a	3.17a	3.27a	0.61a	0.75a

Column means (n = 10) with the same letter are not different $P \leq 0.05$) according to Duncan's multiple-range test.

Reducing sugars = fructose + glucose + others.

Ketone sugars = sucrose + fructans + fructose.

Nematodes increased root starch and decreased reducing sugars. Pruning increased starch, reducing sugars, and ketone sugars. There was an overall increase in concentrations of nonstructural CHO within each treatment from the nonsaline to saline conditions, except for ketone sugars in the untreated controls and roots of seedlings infected with nematodes.

Overall, the effects of nematodes and pruning on osmotically active ions were not different except in magnitude and foliar Na (Tables 8-2, 8-3). Compared with controls, pruning reduced leaf K in both Cleopatra mandarin

TABLE 8-2. Concentrations (% dry weight) of leaf and root osmoticum ions in Cleopatra mandarin seedlings as affected by root pruning and *Tylenchulus semipenetrans* infection with and without low salinity.

Root	K		Cl		Na	
treatment	No salt	Salt	No salt	Salt	No salt	Salt
	Leaf					
Control	1.31a	0.62	0.07a	0.11b	0.09c	0.12b
Nematode	0.51b	0.43b	0.07a	0.17a	0.12b	0.13b
Pruned	0.33c	0.40b	0.08a	0.17a	0.17a	0.19a
	Root					
Control	1.03a	0.82a	0.41a	0.70a	0.11a	0.25a
Nematode	0.90a	0.44b	0.35a	0.60ab	0.14a	0.20b
Pruned	0.74b	0.40b	0.19b	0.52b	0.08b	0.21b

Column means (n = 10) with the same letter are not different $P \leq 0.05$) according to Duncan's multiple-range test.

seedlings and sour orange seedlings. Similarly, *T. semipenetrans* reduced foliar K in both Cleopatra mandarin and sour orange. Pruning and *T. semipenetrans* each

TABLE 8-3. Concentrations (% dry weight) of leaf and root osmoticum ions in sour orange seedlings as affected by root pruning and Tylenchulus semipenetrans infection with and without low salinity.

Root	K		Cl		Na	
treatment	No salt	Salt	No salt	Salt	No salt	Salt
			Leaf			
Control	2.40a	2.19a	0.06a	0.14b	0.10a	0.13a
Nematode	2.25a	1.83b	0.10a	0.18a	0.08a	0.14a
Pruned	1.83b	1.07c	0.06a	0.18a	0.10a	0.10b
			Root			
Control	2.36a	1.75a	o.20a	0.96a	0.41a	0.46a
Nematode	1.57b	1.39b	0.21a	0.76b	0.13b	0.36b
Pruned	1.42b	1.14c	0.15c	0.89ab	0.14b	0.34b

Column means (n = 10) with the same letter are not different $\underline{P} \leq 0.05$) according to Duncan's multiple-range test.

increased foliar Cl in sour orange above controls, and each increased Cl in Cleopatra mandarin leaves. Pruning increased foliar Na in Cleopatra mandarin; whereas nematodes increased foliar Na only in Cleopatra mandarin.

TABLE 8-4. Dry shoot and root weights (g) of Cleopatra mandarin and sour orange as affected by root pruning and Tylenchulus semipenetrans infection with and without low salinity.

Root treatment	Cleopatra mandarin Shoot	Cleopatra mandarin Root	Sour orange Shoot	Sour orange Root
Control	2.77ab	1.48a	6.05a	2.26a
Nematode	2.48b	1.40a	5.92a	1.91b
Pruned	2.97a	0.84b	6.11a	1.18c

Column means (n = 10) with the same letter are not different $P \leq 0.05$) according to Duncan's multiple-range test.

Pruning and nematodes each reduced osmotically active ions in roots. Compared with controls, pruning decreased root K, Cl, and Na in Cleopatra mandarin, and K, Cl, and Na in sour orange. Tylenchulus semipenetrans also reduced root K, Cl, and Na in Cleopatra mandarin and K, Cl, and Na in sour orange. The effects of nematode and root pruning treatments on nonosmotically active ions in both rootstock species were not consistent (Appendices 17,18).

Tylenchulus semipenetrans reduced shoot and root weights below controls, respectively, in Cleopatra mandarin

TABLE 8-5. Tylenchulus semipenetrans per gram fresh root weight of Cleopatra mandarin and sour orange growing grown with and without salinity.

	Cleopatra mandarin		Sour orange	
Life stage	No salt	Salt	No salt	Salt
Female	744a	350b	157a	109a
Juvenile	519a	403a	418a	318a
Egg	2,882a	2,184a	3,847a	2,998a

Within each rootstock, row means (n = 10) with the same letter are not different ($P \leq 0.01$) according to analysis of variance.

and sour orange (Table 8-4). There were no differences between shoot weights of pruned and control plants; whereas root weights in pruned plants were reduced below the controls in Cleopatra mandarin and sour orange.

Compared to sour orange, Cleopatra mandarin seedlings were well infected with nematodes (Table 8-5). Female nematode counts averaged 547 females/g fresh roots (range 325-1,170 females) on Cleopatra mandarin and 133 females/g fresh roots (range 63-332 females) on sour orange. Salinity reduced female densities on Cleopatra mandarin but did not affect other life stages on either rootstock.

Discussion

The pruning technique that we developed precluded contact of the severed areas with soil solution, thus preventing ion fluxes through these areas. The technique also allowed the initiation of salinity just 3 days after pruning without having to wait for pruning wounds to heal. Thus, this facilitated data interpretation by preventing pruned plants from reestablishing prepruned root:shoot ratios prior to measuring ions. Compartmentation minimized the entangling of fibrous roots, thereby avoiding damage to remaining roots during removal of excised halves.

Overall, T. semipenetrans and root pruning had similar effects on osmotically active ions and CHO in roots except the reducing sugars. The nematode data confirmed previous studies which demonstrated that T. semipenetrans parasitism of roots reduces foliar K (Fouche et al., 1977; Milne and Willers, 1979; Van Gundy and Martin, 1961), and root Cl, Na, and (or) K (12,13), and recently increased Cl and (or) Na in leaves. However, most nutrient ions in root pruning studies were measured after the seedlings had restored the prepruned root:shoot ratios (Geisler and Ferree, 1984). This resulted in increased absorption surfaces which either increased or had no effect on foliar nutrient ions compared to those in unpruned controls. Because Cl and Na were never measured in

these pruning studies, this is the first report of the effects of root pruning on the two ions.

The immediate effect of root pruning is a reduced root:shoot ratio. Under this stress, growth is redistributed in favor of root growth, with more photosynthates being diverted towards this effort (Geisler and Ferree, 1984). Photosynthates are translocated to sinks as sucrose, which is osmotically active (Farrar, 1985; Salisbury and Ross, 1985; Waisel, 1972). Recently (Chapter 9) it was demonstrated that T. semipenetrans-infected and root-pruned plants have lower osmotic potential than control plants. In sinks sucrose is hydrolyzed into glucose and fructose molecules which are used in anabolism and catabolism; whereas the excesses are predominantly stored in nonosmotically active forms such as starch, and less so in osmotically active forms such as ketone sugars (Salisbury and Ross, 1985). The accumulation of starch in the remaining roots of pruned plants in this and other studies (Geisler and Ferree, 1984) showed that the rate of sucrose delivery to roots exceeded the combined rates of catabolism and anabolism.

The accumulation of starch in citrus roots under salinity in this, recent, and other (Walker et al., 1984) studies had not been clarified. When glycophytes are initially subjected to salinity stress they undergo 'physiological drought', which is eventually alleviated

through a process referred to as cellular osmotic adjustment (Waisel, 1972). Under nonsaline conditions glycophytes adjust their cellular osmotic potential to the fluctuating external osmotic potential in soil solution by changing the concentrations of K, Na, and Cl in root vacuoles (Waisel, 1972). However, under salinity stress K deficit in glycophytes invariably results (Waisel, 1972; Walker et al., 1984). The higher CHO and lower osmotically active ions in citrus roots, suggest a greater dependency on photosynthetic sucrose for adjusting water potentials in glycophyte cells under salinity. However, because of reduced plant growth under salinity, few glucose units are incorporated into structural forms, so that the excesses are stored as starch.

Generally, the respiration rates of cells under salinity is high (Walker et al., 1984; Williams et al., 1991), which may account for decreased levels of osmotically active sugars in this and other (Chapter 5; Walker et al., 1984) studies. The undamaged cells surrounding those infected by fungi also have high rates of respiration compared to those further from the infected site (Keen and Bruegger, 1977). This may also partly contribute to the reduced osmoticum sugars in nematode infected roots, but the high magnitudes in decrement of these sugars suggest that nematodes consume most of these materials.

The pruning data suggested that _T. semipenetrans_ infection does not alter the partitioning of Cl, Na, and

(or) K exclusively through physical damage of cell membranes (O'Bannon and Esser, 1985; Tarjan and O'Bannon, 1987). In shoot pruned citrus, K was redistributed from roots to shoots, resulting in high and low K in leaves and roots, respectively (Swietlik, 1986). However, under either nematodes or pruning, K reductions in roots and leaves occurred concurrently, suggesting that K leaches out of the plant instead of being redistributed from organ to organ. Also, because there were no differences in shoot weights of pruned and control plants, it appears that the suppression of shoot growth under salinity occurs as a result of Cl and Na accumulation in leaves, and not vice versa (Swietlik, 1986).

The pruning data supported our hypothesis which proposes that the altered partitioning of Cl, Na, and (or) K in citrus is related to high CHO in roots. The reduction of Cl, Na, and (or) K in citrus roots appears to be a common phenomenon in _T_. _semipenetrans_ infected in recent and other (Labanauskas et al., 1965) studies, VAM inoculated (Hartmond et al., 1987), and salinity stressed (Walker et al., 1984) plants, and is related to elevated root CHO (Koch and Johnson, 1984; Williams et al., 1991). In contrast, stresses which reduce CHO in roots, such as ringing and lemon collapse, increase Na in roots (Rodney et al., 1956).

Based on the pruning data, the mechanism whereby T. semipenetrans reduces osmoticum ions in roots appears to be a four-step process: (1) the nematodes reduce the root system through parasitism, (2) the plant then diverts more photosynthates (sucrose) to roots in an attempt to restore the root:shoot ratio, (3) in roots, sucrose and its hydrolyzed units reduce water potential, and finally, (4) as a measure to counteract the sucrose-induced declining water potential, root cells excrete osmotically active ions (Cl,Na,K) into the apoplasm. Also, less Cl and Na are absorbed by root cells, which are carried via the transpiration stream and accumulate in the leaves.

Generally, the concentrations of ions in root symplasm are several thousand-folds higher than those in soil solution, whereas those in apoplasms are equal to those in soil solution (Salisbury and Ross, 1985; Waisel, 1972). Under NaCl salinity Cl and Na in soil solution are high (Bohn et al., 1985), thus excreting these ions into apoplasm and (or) not absorbing them from apoplasm, further elevates the levels of the two ions in the apoplasm. The two ions are eventually transported into the xylem vessels and carried via the transpiration stream to shoots, where they accumulate, with leaf abscission being the sole method by which glycophytes rid themselves of excess Cl and (or) Na. However, because levels of K in soil solution are low (Bohn et al, 1985; Salisbury and Ross, 1985), our model suggests

that when K is excreted into apoplasm, the ion leaches out into soil solution, where it becomes unavailable compared to Cl and Na. However, K is required for activating starch synthase, which hydrolyzes sucrose into glucose and fructose (Salisbury and Ross, 1985). Because of the resulting K deficits in roots, foliar K is mobilized to roots, where its role in catalyzing starch synthase prevents excess accumulation of sucrose in roots, which would otherwise counteract the role of ion excretion. However, upon arrival to roots, as more sucrose from photosynthesis arrives, this K is not immune to excretion, resulting in both foliar and root K deficits. When K levels in soil solution are augmented through fertilizers (Graham and Syvertsen, 1989), upon excretion into apoplasm, K follows the same route as Cl and Na. However, if nematode densities are not reduced, the continual increase of root CHO may prevent K absorption by root cells; whereas accumulated foliar Na may prevent K from occupying the sites where it catalyzes reactions in leaves. Fouche et al. (1977) showed that increasing soil K without reducing the nematode levels was not beneficial to trees.

CHAPTER 9
OSMOTIC POTENTIAL, OSMOTICUM IONS, TRANSPIRATION, AND CO_2 ASSIMILATION IN SOUR ORANGE SEEDLINGS AS AFFECTED BY TYLENCHULUS SEMIPENETRANS AND MECHANICAL ROOT PRUNING

Introduction

Infection of citrus by *Tylenchulus semipenetrans* Cobb produces characteristic changes in the partitioning of chloride (Cl), sodium (Na), and potassium (K) in citrus leaf and fibrous root tissues (Chapters 5,6,7,8; Fouche et al., 1977; Labanauskas et al., 1965; Milne and Willers, 1979; Van Gundy and Martin, 1961). Overall, *T. semipenetrans* infection of roots reduces K in leaves along with Cl, Na, and K in roots; whereas it increases the accumulation of Cl and Na in leaves (Chapter 5). Mechanical root pruning induced similar effects when seedlings were harvested prior to restoring the prepruned root:shoot ratios (Chapter 8). Root pruning and *T. semipenetrans* each reduces fibrous roots, which are required for ion uptake and water absorption (Atkinson, 1980).

Generally, root pruning decreases shoot growth (Richards and Rowe, 1977), CO_2 assimilation (Taylor and Ferree, 1981), and transpiration (Stansell et al., 1974). However, root pruned citrus seedlings had higher starch and reducing sugars in the remaining roots; whereas *T.*

semipenetrans-infected roots also had higher starch but lower reducing sugars than the controls (Chapter 8). High humidity induced nutrient element deficiencies in tomato (Adams, 1991), suggesting a relationship between nutrient accumulation in leaves and transpiration rates. The accumulation of root starch and foliar Cl and Na in T. semipenetrans-infected and root-pruned seedlings in previous studies (Chapters 5,8) suggested that either stress may increase photosynthesis (CO_2 assimilation) and (or) transpiration rates. To test these hypotheses, the effects of T. semipenetrans and root pruning on photosynthesis and whole-plant transpiration (WPT) were periodically measured in sour orange seedlings grown in a temperature regulated greenhouse. Since reduced osmotic potential (π) in nematode infected plants may account for the altered partitioning of Cl, Na, and K, the effects of T. semipenetrans and root pruning on osmotic potential were also measured.

Materials and Methods

Taproots of 6-month-old sour orange (Citrus aurantium L.) seedlings were vertically split into two halves starting from the tip to 10 cm above the soil surface. The joint in the stem was secured with Parafilm M (American Co., Greenwich, CT). Each plant was transplanted into two 15-cm-diam clay pots containing a soil mix of 3:1 (v/v) steamed

autoclaved sand (97% sand, 2% silt, 1% clay; pH 7.1, 2% organic matter) and PRO-MIX (Premier Brands, Inc., Stamford, Canada). The root systems were allowed to develop for 20 to 26 weeks in each pot.

The experimental treatments consisted of nematodes, root pruning, and controls. Seedlings were selected for uniformity three months after transplanting, and arranged on a greenhouse bench in a randomized complete block-factorial design with seven replicates. Each pot was initially irrigated with 100 ml tap water every day until the first shoot flush, and then with 250 ml water every second day. Each pot was fertilized weekly with 5 g 20:20:20 mixture ($N:P_2O_5:K_2O$) per liter of water at the same rate as irrigation water, and biweekly with a 25% Hoagland's solution (Hoagland and Arnon, 1950) to supply micronutrients. Ambient greenhouse temperatures averaged 28 C maximum (range 26-30 C) and 24 C minimum (range 20-27 C).

The nematode treatment was initiated 12 weeks after transplanting. Nematode juveniles for inoculum were collected, prepared, disinfested, and inoculated three times with a total of 210,000 juveniles/pot (382 nematodes/100 cm^3) over a 3-day interval as described previously (Chapter 7). The control and pruned treatments were inoculated with an equal amount of nematode filtrate (25-μm-pore sieve) to establish in their rhizosphere any microbes associated with nematodes. Pruning was imposed by excising one of the root

half systems 1 cm below the joint eight weeks after inoculation. Because the pruned halves were randomly selected, this presumably removed half of the total root system while leaving one-half undisturbed. The concentrations of fertilizers for control and nematode treatments were reduced by half after pruning so that all plants received equal nutrient concentrations regardless of rooting volume.

One mature leaf/plant was sampled between 6h00 and 6h15 on days 0, 14, 28, and 42 after pruning to measure leaf π. Five discs/leaf were sealed in a scintillation vial (Fisher) and stored at -80 C until π was measured. The leaf remnants were dried at 70 C for 48 hours, weighed, and analyzed (Rhue and Kidder, 1983) for Cl using a Haake Chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ).

Net CO_2 assimilation rates were measured on one fully expanded developed leaf/plant using a Licor portable photosynthesis system 6,200 (LI-COR, Inc., Lincoln Nebraska, NE) between 9h00 and 11h00 on days 0, 14, and 28 after pruning. The system was equipped with a 1 liter cuvette and also computed stomatal conductance, transpiration, leaf temperature, and internal CO_2.

Whole-plant-transpiration (WPT) was measured biweekly using gravimetric methods on 0, 2, 14, 28, and 42 days after pruning. Briefly, both pots/plant were placed into a single white plastic bag in the evening prior to taking

measurements. The following morning (8h00 to 8h30) the bags were firmly tied around the base of the stem to prevent evaporation from the soil, and weighed. Pots were reweighed at 18h00-18h30 and the difference between the morning and evening weights was the total water loss/plant. Four additional plants (2 with and 2 without nematodes) were harvested, their individual leaf lengths (L) and widths (W) measured, and their leaf areas measured using a leaf area meter (LI-COR, Inc.). The linear regression equation,

$$\text{Leaf area (cm}^2\text{)} = -0.188 + 0.560(\text{WxL}), \ (r^2 = 0.95)$$

was used to express WPT on a total leaf area basis.

Plants were harvested 44 days after pruning. Roots were rinsed in tap water, excess water removed between tissue papers, and then stored in sealed plastic bags at 5 C. Root length/plant was estimated using the grid intercept method (Newman, 1966). About 0.5 g of fresh roots/plant was stored in scintillation vials at -80 C. Frozen root and leaf samples were thawed while still in sealed vials at 5 C for 12 hours. Tissue sap was pipetted onto a paper disc and its π measured using a Wescor vapor pressure osmometer (Wescor Co.) which was calibrated using 0.01, 0.05, 0.10, 0.50, and 1.00 molal NaCl solutions.

Nematodes were extracted from 1 g fresh roots/plant, stained, and counted as described previously (Chapter 3). The remaining roots and shoots were dried at 70 C for 48 hours and weighed. Roots and leaves were ground in a Wiley

mill to pass a 375-μm-pore sieve, and Na and K were analyzed (Rhue and Kidder, 1983) by an inductively coupled plasma emission spectrometer (Perkin Elmer, Co., Norwalk, CT) and Cl was analyzed using a Haake Chloridometer.

Treatment effects were evaluated using analysis of variance (ANOVA). The nematode data were transformed into ln(x+1) prior to ANOVA to homogenize the variance (Little and Hills, 1975). Data with significant ($P \leq 0.05$) F-statistic were separated using Duncan's multiple-range test. Unless stated otherwise, the nematode and root pruning data are discussed relative to the untreated control, and the treatments were not different at $P \leq 0.10$.

Results

Nematode. Nematode infection averaged 518 females/g fresh roots (range 498-916). The juveniles and eggs per g fresh roots averaged 471 (range 337-816) and 8,117 (range 6,659-10,925), respectively.

Osmotic potential (π). One day prior to pruning, nematode infected seedlings had 34% lower π than the controls, whereas π of pruned and control plants did not differ (Table 1). The leaf π for pruned plants on days 14,

TABLE 9-1. Leaf and root osmotic potentials (MPa) of sour orange seedlings as affected by root pruning and *Tylenchulus semipenetrans* infection.

	Leaf				Root
	Days after pruning				
Treatment	0	14	28	42	44
Control	-0.19a	-0.20c	-0.19c	-0.22c	-0.25c
Nematode	-0.26b	-0.31b	-0.36b	-0.40b	-0.28bc
Pruned	-0.18a	-0.40a	-0.51a	-0.59a	-0.30a

Column means (n = 7) with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

28, and 42 was less than controls by 110%, 162%, and 189%, respectively. The leaf π of nematode infected plants on those days were 61%, 83%, and 95% below the controls. Similarly, by harvest root π due to pruning and nematodes were, respectively, 21% and 12% ($P \leq 0.10$) below the controls.

Osmoticum ions. Nematode infection resulted in high Cl (317%) and Na (82%) in leaves, but low K (41%) in leaves (Table 2). In roots, nematode infection decreased Cl (17%), Na (26%), and K (43%). Pruning had no effect on osmoticum

TABLE 9-2. Leaf and root osmoticum ions (% dry weight) of sour orange seedlings as affected by root pruning and *Tylenchulus semipenetrans* infection.

		Sampling	Root treatment		
Tissue	Element	time†	Control	Nematode	Pruned
Leaf	Cl	0	0.06b	0.21a	0.05b
	Cl	14	0.05b	0.29a	0.27a
	Cl	28	0.08c	0.32b	0.43a
	Cl	44	0.06c	0.38b	0.54a
	K	44	2.28a	1.39b	2.30a
	Na	44	0.35a	0.26b	0.38a
Root	Cl	0	0.52a	0.43b	0.69a
	K	44	2.28a	1.30b	2.30a
	Na	44	0.35a	0.26b	0.38a

Within the same sampling time, row means (n = 7) with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

† Days after initiating the pruning treatment.

ions in roots or K in leaves, but increased Na (250%) and Cl (438-800%) in leaves. Generally, pruning did not affect nonosmotically active ions except reducing Ca (5%, $P \leq 0.10$)

TABLE 9-3. Shoot and root weights (g), shoot height (cm), root length (cm), and leaf area (cm^2) of sour orange seedlings as affected by root pruning and *Tylenchulus semipenetrans* infection.

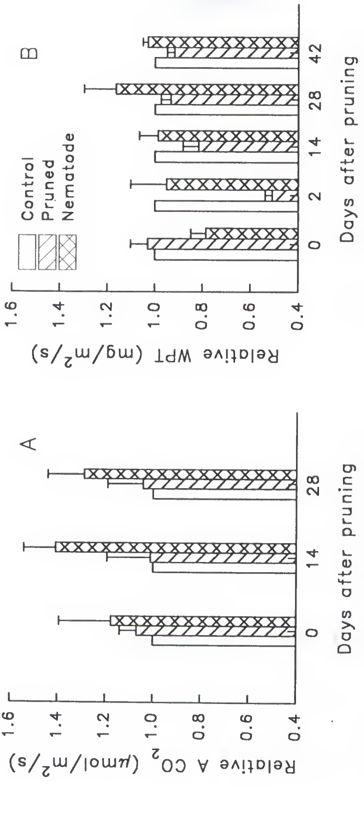
Variable	Sampling time	Root treatment Control	Nematode	Pruned
Shoot weight	44	11.10a	8.63b	7.99c
Root weight	44	3.13a	2.32b	2.12b
Shoot height	44	70.00a	62.00b	56.00c
Root length	44	2,925.00a	2,229.00b	2,209.00b
Leaf area	14	390.00a	368.00a	338.00a
	28	590.00a	590.00a	482.00b
	42	915.00a	891.00ab	773.00b

Row means (n = 7) with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

in leaves (data not shown). The nematodes reduced P (11%) and Cu (32%) in leaves, and also reduced P (25%) in roots (data not shown).

CO_2 assimilation. The only treatment that affected photosynthesis rates was nematodes (Fig. 1A). *Tylenchulus semipenetrans* increased photosynthesis on days 0 (29%) and 14 (41%), and 28 (22%). Root pruning had no effect on

FIGURE 9-1. Relative effects of _Tylenchulus_ _semipenetrans_ and mechenical root pruning on A) Photosynthesis, and B) Whole-plant-transpiration rates on sour orange seedlings.


A
Relative A CO_2 (μmol/m^2/s)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0
14
28
Days after pruning
B
Relative WPT (mg/m^2/s)
Control
Pruned
Nematode
0
2
14
28
42
Days after pruning

photosynthesis rates regardless of date. The nematodes increased stomatal conductance on day 14 (54%), while pruning increased (7%, $\underline{P} \leq 0.10$) WUE on day 28 (data not shown).

Whole plant transpiration. Mean WPT under nematodes was 18% below the controls prior to pruning (Fig. 1B). However, on days 14 and 42 nematodes had no effect on transpiration, but increased it (16%) on day 28. Root pruning had no effect on transpiration on day 0, but reduced transpiration (50%) two days after pruning, and then transpiration rates slowly recovered so that on day 14 transpiration was 18% below the controls, and on day 28 it did not differ from that of the controls. However, on day 42 transpiration had dropped by 10% below the controls.

Growth characteristics. The nematodes limited shoot (22%) and root (26%) weights, and shoot (11%) height (Table 3). Pruning also suppressed shoot (28%) and root (32%) growths, and top (20%) heights. However, the root:shoot ratios did not differ 44 days after pruning. The leaf areas also did not differ among the treatments on day 14, but were lower for pruned plants on days 28 and 42. Nematodes and pruning each reduced root length by 24% below the control.

Discussion

Tylenchulus _semipenetrans_ and mechanical root pruning consistently reduced foliar osmotic potential under the conditions of this study. Other nematode species, _Globodera_ _rostochiensis_ (Trudgill and Cotes, 1983), _Meloidogyne_ _hapla_ (Wilcox and Loria, 1986), and _M_. _javanica_ (Wallace, 1974), also decreased foliar osmotic potential on different plant cultivars; whereas _Pratylenchus_ _penetrans_ (Kotcon and Loria, 1986) had no effect on osmotic potential in leaves. This is, however, the first report on the decrement of osmotic potential in leaves by root pruning. Both treatments, as in previous studies (Chapter 8), increased Cl and Na in leaves. Because osmotically active sugars did not previously accumulate in leaves of seedlings infected with _T_. _semipenetrans_ (Chapter 5,8), increased Cl and Na may account for the reduction in leaf osmotic potential. The higher Cl and Na in the remaining roots under pruning, in addition to accumulated reducing sugars, accounted for the reduced osmotic potential in roots.

The effects of _T_. _semipenetrans_ on Cl, Na, and K were consistent with those observed previously. However, the pruning effects on root osmoticum ions and leaf K in this study are not comparable with those in the previous study because then plants were harvested prior to restoring their prepruned root:shoot ratios. Others (Humphries, 1958b;

Richards and Rowe, 1977) also demonstrated that when pruned plants were harvested after restoring their prepruned root:shoot ratios, pruning would either increase or not interact with nutrient ions in leaves. This is reasonable because although all parts of the root system absorb nutrients, the apices are the most active for immobile elements such as Ca (Atkinson, 1980) and most likely, for other ions as well. Also, the presence of more lateral roots after attaining the prepruned root:shoot ratios may increase the absorption surface. This relates to the observed higher levels of nutrients in one pruning study (Richards and Rowe, 1977), and the increasing trends of osmoticum ions in both leaves and roots in this study.

Generally, nematodes reduce photosynthesis (Loveys and Bird, 1973; Meon et al., 1978; Wilcox and Loria, 1986). However, the responses of photosynthesis to nematode infection depended on plant age, plant and nematode species, inoculum densities, and measurement date relative to inoculation date (Wallace, 1974). Wallace (1974) observed that 500 and 1,000 _M. javanica_ juveniles/pot increased photosynthesis on tomato plants, 250 specimens had no effect, while 2,000 reduced photosynthesis more than 1,000 except on old plants. Mean photosynthesis on _T. semipenetrans_ plants remained above the controls regardless of date, probably due to the high infection levels. This observation, supported by the accumulation of root starch in

previous studies (Chapters 5,6,7,8), supports the hypothesis that this parasite may be a stronger "apparent" sink for photosynthates. Although carbohydrates also accumulated in the remaining roots under pruning, mean photosynthesis for pruned and control plants did not differ at any date. Others (McDavid et al., 1973; Stansell et al., 1974; Stenphens, 1964) reported reduced photosynthesis soon after root pruning, followed by slow recovery as the prepruned root:shoot ratios were approached.

The observed oscillations in transpiration rates under T. semipenetrans reflected the inconsistencies of data on interactions of nematodes and transpiration rates (Meon et al., 1978; Wilcox and Loria, 1986). Responses of transpiration to nematode infection, as with photosynthesis (Wallace, 1974), may also depend on nematode population levels and the relative times of inoculation variable and measurement. Because the infection potential of T. semipenetrans is lower than that of other nematode species, our extended duration from inoculation to measuring transpiration, might also have caused fluctuations of nematode levels, resulting in changes of transpiration rates over the 42-day period.

The strong effects of root pruning on transpiration agree with those in other studies (Stansell et al., 1974). The 50% reduction in transpiration soon after root pruning suggests that there may be a strong linear relationship

between the absorptive surface area and transpiration rates. Because mean transpiration values for all treatments were not different on day 28, it was predicted that the pruned plants had attained the prepruned root:shoot ratios, and that the pruned root system grew out of pruning stress so that there would be no longer any effect of pruning on transpiration. However, at the end of the 14-days interval, pruned plants had decreased transpiration (10%) compared to the controls, suggesting that the functional physiology of pruned and control plants were still out of phase. The reduced transpiration and increased foliar Cl and Na of root-pruned seedlings demonstrated that the accumulation of the two ions in leaves was not related to higher transpiration rates. Adams (1991) demonstrated that low transpiration rates may reduce the accumulation of essential nutrient elements such as K and Ca in leaves.

Tylenchulus semipenetrans, as in the previous studies (Chapters 5,7,8), reduced shoot and root weights, and top heights. Similar effects occurred under pruning, which agree with other studies (Alexander and Maggs, 1971; Richards and Rowe, 1977). In root-pruned plants, however, shoot growth is depressed in favor of root growth, as demonstrated by the restored root:shoot ratios. In nematode-infected seedlings, the intensifying nematode stress over time, appears to negate the advantages of diverting assimilates belowground for root regeneration.

The increased accumulation of starch in roots of nematode-infected and root-pruned plants suggests that assimilate availability is not the factor limiting growth under these two treatments.

Cytokinins, synthesized in feeder root apices, enhance cell elongation, required for plant growth (Skene, 1975). The imbalances in hormonal supply, caused when root apices are mechanically and (or) parasitically reduced, could account for the reduced rates of incorporating nonstructural carbohydrates into structural forms, as demonstrated by the accumulation of starch in roots of previous studies (Chapters 5,6,7,8). Also, because plant growth is inversely proportional to cellular osmotic potential (Hsaio, 1973), in addition to specific ion toxicities, excess reduction in osmotic potential could also limit plant growth.

CHAPTER 10
SUMMARY AND CONCLUSIONS

Worldwide, the availability of high quality water for agricultural irrigation is decreasing, while salinity in irrigation water is increasing. Citrus is sensitive to high salinity and also to the nematode, Tylenchulus semipenetrans Cobb. There is currently no commercially acceptable citrus rootstock that is both tolerant to salinity and resistant to T. semipenetrans. Salt tolerance in citrus is defined as the ability of roots to exclude excess Cl and (or) Na from shoots. Resistance to T. semipenetrans is expressed as the ability of roots to suppress female development and egg production.

Cyclic salinity is typical of field conditions, particularly in regions with wet and dry seasons. During dry seasons, supplemental irrigation with poor quality water accumulates salts in the upper soil horizons where a high percentage of feeder roots and T. semipenetrans population densities occur. Relative to continuous salinity or nonsaline conditions, cyclic salinity in the root zone increased T. semipenetrans female and egg counts in the greenhouse. Cyclic salinity also reduced resistance to the

citrus nematode in citrus rootstock seedlings representing a wide range of _T_. _semipenetrans_-resistant germplasm.

Tylenchulus semipenetrans infection reduced salt tolerance in citrus rootstock seedling representing a wide range of salt tolerant germplasm in the greenhouse. Relative to untreated controls, infected seedlings had physiologically toxic levels of Cl and Na in leaves; whereas infected roots had lower levels of Cl and Na than the nematode-free roots. Also, the nematode consistently reduced K in both roots and leaves, along with foliar Cu. However, nematode effects on Ca, Mg, P, Fe, Mn, and Zn were variable among the six rootstock species. Nematode-infected roots had consistently higher concentrations of starch than the noninfected control roots.

Tylenchulus semipenetrans also increased Cl and Na but reduced K in leaves of citrus replants and mature trees under field conditions. Nematode-infected replants also had lower foliar Cu, Zn, and Mn along with root Cl, Na, and K. The data supported the hypothesis that this nematode induces slow decline of citrus by upsetting nutrient balances. Additionally, the data suggested that Cl and Na toxicities are integral components in _T_. _semipenetrans_-induced diseases of citrus. In fact, this study demonstrated that clinical symptoms of slow decline can be directly linked to specific nutrient element deficiencies and (or) specific ion toxicities.

Chloride, Na, K, and sucrose each is osmotically active in plant cells. Periodic measurements demonstrated that relative to the controls, _T_. _semipenetrans_-infected plants had lower osmotic potential. This observation supported the hypothesis that nematode-infection results in imbalances of osmotically active ions in citrus through osmotic potential imbalances.

A proposed mechanism is that when root systems are reduced, more assimilates (sucrose) are diverted belowground for root regeneration. Because sucrose is osmotically active, it reduces the osmotic potential of root cells, resulting in increased osmosis and subsequently, higher turgor pressure in relatively young regrowing root systems. However, turgor pressure in root cells is maintained relatively constant, regardless of the osmotic potential of the substrate (Zimmermann et al., 1992).

To maintain turgor pressure within a constant range, as more assimilates are diverted to the reduced root systems, excess monosaccharides are converted to nonosmoticum forms such as starch. This, together with increased efflux of osmotically active ions into the apoplasm, maintains turgor pressure relatively constant. However, once in the apoplasm, K is leached out of the root because of its inherent low concentrations in soil solution; whereas Cl and Na accumulate due to their relatively high concentrations in soil solution. Eventually, Cl and Na cross the endodermis

into the transpiration stream, through which they are transported to leaves, where they accumulate.

Inducing carbohydrate accumulation in roots through mechanical root pruning enhanced the characterization of mechanisms involved in salinity-nematode interactions. Root pruning simulated the effects of T. semipenetrans on the partitioning of Cl, K, and Na when seedlings were harvested prior to restoring the prepruned root:shoot ratios. However, after this ratio was reestablished, foliar K and root Cl, Na, and K status became similar to those in control plants, whereas Cl and Na in leaves remained high in previously root pruned plants. The continued high levels of foliar Cl and Na suggested that once the plant accumulated these ions in leaves, their transport out is limited. This suggested that citrus might only rid itself of excess foliar Cl and Na through defoliation.

Increased starch in nematode-infected roots suggested that T. semipenetrans created an apparent photosynthate sink. This was supported by the fact that T. semipenetrans infection resulted in increased photosynthesis compared to controls. However, pruning and subsequent root regrowth had no effect on photosynthesis.

The effects of nematodes on whole-plant-transpiration were variable. Relative to the untreated controls, root pruning reduced transpiration until the prepruned root:shoot ratios were reestablished. The higher accumulation of Cl

and Na in leaves of pruned seedlings, in spite of the reduced transpiration rates, suggested that accumulation of the two ions in leaves was independent of transpiration rates.

Under field conditions, populations densities of T. semipenetrans are often concentrated in the upper soil horizons; whereas salinity often becomes more concentrated with soil depth. When salinity and nematodes were spatially separated in split-root studies, salinity increased nematode levels above those when salinity occurred concomitantly with nematodes or in the absence of salinity. In contrast, when combined with salinity, the citrus nematode resulted in increased foliar Cl and Na, more accumulated starch in roots, more imbalanced nutrient ions, and more reduced plant growth. This supported the view that the effects of T. semipenetrans parasitism of roots are more severe under salinity.

Validation of data in this study would result in numerous practical applications. For instance, because Cl and Na in citrus leaves tend to be localized upon accumulation, these may serve as predictive models for yield loss due to T. semipenetrans damage. Under microjet irrigation, only a portion below the canopy is irrigated, thus resulting in pockets of salinity. Thus, studying the distribution of T. semipenetrans under the canopy of trees irrigated with microjet systems could clarify, inter alia,

the time and (or) exact location of nematicidal placement, thus improving the efficacy of nematicides. Because citrus acreage under sewage water irrigation in Florida is increasing, and because this water is inherently high in Na and Cl ions, reciprocal interactions of sewage water and *T. semipenetrans* should be evaluated.

The hypothesis that increased nonstructural root carbohydrates displace osmotically active ions, may find general application in plant stress and nutrient element research.

To summarize, salinity increases population densities of *T. semipenetrans* when the two factors are spatially separated; whereas the nematode reduces tolerance to salinity when the two factors are interacting directly. High concentrations of nonstructural carbohydrates in roots increase the efflux of osmotically active ions; and this is the underlying mechanism by which *T. semipenetrans* induces slow decline and replant disorders of citrus.

APPENDICES

APPENDIX 1. Effects of salinity on Tylenchulus semipenetrans juvenile eclosion.

Experiment	N	T (C)	Salinity treatment: Control	Salinity treatment: Salinity
Petri dish	4	26	183	15 **
Sandy soil	10	27	238	128 *
Organic mix	10	27	415	93 **

** Significant at $P < 0.01$, * $P < 0.05$.

Organic mix = Sand:PRO-MIX BX (1:1, v/v).

Approximately 300 eggs in petri dishes, and 1,000 eggs in sand and organic mix.

Salt = 50 mols NaCl/m^3 H_2O: petri dishes over 48 hours, in fallow over 7 days with every other day irrigation.

APPENDIX 2. Foliar chloride (%) with and without Tylenchulus semipenetrans infection 2 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	0.06	0.07	0.14	0.40
Rangpur	T	0.06	0.06	0.15	0.30
Sour orange	M	0.05	0.06	0.16	0.40
Rough lemon	M	0.05	0.07	0.17	0.50
Sweet lime	S	0.04	0.07	0.12	0.35
Volkamer	S	0.05	0.07	0.17	0.40

Source of variation	df	Total Treatment Variation SS	Percentage
Rootstock	5	0.13 †	1.84
Salinity	1	1.38 **	19.87
Nematode	1	4.08 **	58.69
R x S	5	0.11 ns	1.56
R x N	5	0.07 ns	1.07
S x N	1	1.14 **	16.34
R x S x N	5	0.05 ns	0.65
Error	335	4.89	

**Significant at $P \leq 0.01$, † $P \leq 0.10$; ns = not significant
at $P \leq 0.10$.

APPENDIX 3. Foliar copper (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	47	21	28	25
Rangpur	T	27	24	26	23
Sour orange	M	34	22	28	26
Rough lemon	M	27	23	24	20
Sweet lime	S	23	21	24	21
Volkamer	S	35	20	30	18

Source of variation	df	Total Treatment Variation SS	Total Treatment Variation Percentage
Rootstock	5	1,292 †	18.52
Salinity	1	295 ns	4.23
Nematode	1	2,015 **	28.88
R x S	5	304 ns	4.36
R x N	5	1,791 *	25.67
S x N	1	507 *	7.27
R x S x N	5	772 ns	11.07
Error	335	1,453	

**Significant at $P \leq 0.01$, *$P \leq 0.05$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 4. Root copper (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	40	40	34	33
Rangpur	T	52	43	41	40
Sour orange	M	34	33	32	34
Rough lemon	M	61	58	28	35
Sweet lime	S	36	53	42	48
Volkamer	S	27	27	32	24

Source of variation	df	Total Treatment Variation SS	Percentage
Rootstock	5	8,297.54 **	48.13
Salinity	1	2,140.01 **	12.93
Nematode	1	18.13 ns	0.00
R x S	5	4,660.59 **	26.82
R x N	5	1,322.84 ns	7.68
S x N	1	0.05 ns	0.00
R x S x N	5	676.05 ns	3.89
Error	335	50,901.63	

**Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

APPENDIX 5. Foliar calcium (%) with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	2.22	1.88	1.82	1.92
Rangpur	T	2.00	1.84	1.53	1.41
Sour orange	M	1.93	1.66	1.67	1.71
Rough lemon	M	1.52	1.66	2.11	1.61
Sweet lime	S	1.97	1.55	1.68	1.53
Volkamer	S	1.80	1.60	1.92	1.44

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	2.17 ns	27.18
Salinity	1	0.34 ns	4.51
Nematode	1	1.44 *	17.81
R x S	5	2.23 ns	28.35
R x N	5	0.29 ns	3.19
S x N	1	0.01 ns	0.00
R x S x N	5	1.51 ns	19.12
Error	168	48.63	

**Significant at $P \leq 0.01$, *$P \leq 0.05$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 6. Root calcium (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	0.58	0.65	0.79	0.63
Rangpur	T	0.68	0.75	0.81	0.69
Sour orange	M	1.65	0.96	1.91	0.88
Rough lemon	M	0.68	0.61	0.76	0.63
Sweet lime	S	0.71	0.77	0.76	0.79
Volkamer	S	0.58	0.49	0.59	0.52

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	13.16 **	66.23
Salinity	1	0.14 ns	0.71
Nematode	1	1.51 **	7.62
R x S	5	0.04 ns	0.18
R x N	5	4.62 **	23.22
S x N	1	0.22 ns	1.09
R x S x N	5	0.19 ns	0.95
Error	168	14.50	

**Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

APPENDIX 7. Foliar magnesium (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	0.33	0.26	0.23	0.28
Rangpur	T	0.19	0.22	0.17	0.17
Sour orange	M	0.28	0.26	0.21	0.23
Rough lemon	M	0.21	0.27	0.24	0.20
Sweet lime	S	0.20	0.19	0.18	0.15
Volkamer	S	0.25	0.20	0.21	0.16

Source of variation	df	Total Treatment Variation SS	Total Treatment Variation Percentage
Rootstock	5	0.21 **	62.21
Salinity	1	0.05 **	15.10
Nematode	1	0.00 ns	0.00
R x S	5	0.01 ns	1.45
R x N	5	0.01 ns	1.45
S x N	1	0.00 ns	0.00
R x S x N	5	0.06 †	15.98
Error	168	0.95	

**Significant at $P \leq 0.01$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 8. Root magnesium (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	0.23	0.29	0.22	0.21
Rangpur	T	0.18	0.17	0.19	0.17
Sour orange	M	0.15	0.13	0.13	0.15
Rough lemon	M	0.18	0.19	0.14	0.17
Sweet lime	S	0.15	0.16	0.16	0.15
Volkamer	S	0.18	0.16	0.17	0.16

Source of variation	df	Total Treatment Variation: SS	Total Treatment Variation: Percentage
Rootstock	5	0.18 **	80.12
Salinity	1	0.01 ns	3.11
Nematode	1	0.00 ns	0.00
R x S	5	0.01 ns	4.91
R x N	5	0.01 ns	4.91
S x N	1	0.00 ns	0.00
R x S x N	5	0.02 ns	5.91
Error	168	0.46	

**Significant at $P \leq 0.01$; ns = not significant at $P \leq 0.10$.

APPENDIX 9. Foliar zinc (%) with and without *Tylenchulus semipenetrans* infection 4 weeks after salinization.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	74	65	64	75
Rangpur	T	59	62	51	51
Sour orange	M	52	42	42	53
Rough lemon	M	56	62	53	56
Sweet lime	S	55	54	53	45
Volkamer	S	56	45	54	46

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	10,050.17 **	70.49
Salinity	1	540.02 ns	3.79
Nematode	1	70.08 ns	0.49
R x S	5	623.85 ns	4.38
R x N	5	1,120.54 ns	7.86
S x N	1	325.52 ns	2.28
R x S x N	5	1,526.98 ns	10.71
Error	168	63,112.75	

**Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

APPENDIX 10. Root zinc (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	210	237	152	131
Rangpur	T	189	173	165	162
Sour orange	M	198	114	209	105
Rough lemon	M	246	128	162	102
Sweet lime	S	174	148	173	121
Volkamer	S	172	85	116	85

Source of variation	df	Total Treatment Variation: SS	Total Treatment Variation: Percentage
Rootstock	5	82,271.11 *	22.63
Salinity	1	48,453.36 **	13.33
Nematode	1	106,783.59 **	29.39
R x S	5	38,755.92 ns	10.66
R x N	5	66,419.73 †	18.27
S x N	1	1,171.74 ns	0.32
R x S x N	5	19,637.60 ns	5.40
Error	168	1,083,449.25	

**Significant at $P \leq 0.01$, *$P \leq 0.05$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 11. Foliar manganese (%) with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	41	44	46	49
Rangpur	T	38	37	27	32
Sour orange	M	35	30	37	32
Rough lemon	M	37	36	43	40
Sweet lime	S	40	39	37	37
Volkamer	S	34	26	51	32

Source of variation	df	Total Treatment Variation SS	Total Treatment Variation Percentage
Rootstock	5	3,612.09 **	46.77
Salinity	1	190.01 ns	2.46
Nematode	1	497.30 †	6.44
R x S	5	1,789.59 *	23.17
R x N	5	1,254.42 ns	16.24
S x N	1	0.26 ns	0.00
R x S x N	5	379.34 ns	4.92
Error	168	27,272.38	

**Significant at $P \leq 0.01$, *$P \leq 0.05$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 12. Root manganese (%) with and without Tylenchulus semipenetrans infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	222	237	161	112
Rangpur	T	171	141	199	127
Sour orange	M	290	108	274	111
Rough lemon	M	348	84	252	94
Sweet lime	S	233	120	198	161
Volkamer	S	230	75	195	69

Source of variation	df	Total Treatment Variation SS	Percentage
Rootstock	5	69,594.21 ns	6.78
Salinity	1	31,186.51 †	3.05
Nematode	1	593,963.76 **	57.89
R x S	5	57,141.46 ns	5.57
R x N	5	226,829.71 **	22.11
S x N	1	5,053.26 ns	0.49
R x S x N	5	42,166.09 ns	4.11
Error	168	1,411,544.88	

**Significant at $P \leq 0.01$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 13. Foliar phosphorus (%) with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	0.71	0.40	0.29	0.29
Rangpur	T	0.26	0.32	0.25	0.24
Sour orange	M	0.26	0.21	0.19	0.22
Rough lemon	M	0.37	0.24	0.32	0.32
Sweet lime	S	0.26	0.29	0.26	0.30
Volkamer	S	0.25	0.21	0.29	0.2

Source of		Total Treatment Variation	
variation	df	SS	Percentage
Rootstock	5	0.79 **	42.83
Salinity	1	0.10 ns	5.13
Nematode	1	0.06 ns	3.26
R x S	5	0.49 **	26.37
R x N	5	0.20 ns	10.41
S x N	1	0.07 ns	4.29
R x S x N	5	0.19 ns	10.11
Error	168	15.62	

**Significant at $\underline{P} \leq 0.01$; ns = not significant at $\underline{P} \leq 0.10$.

APPENDIX 14. Root phosphorus (%) with and without *Tylenchulus semipenetrans* infection 4 weeks after salinity.

		Nonsaline		Low salinity	
Rootstock	Class	Control	Nematode	Control	Nematode
Cleopatra	T	0.17	0.22	0.24	0.15
Rangpur	T	0.15	0.16	0.14	0.14
Sour orange	M	0.13	0.12	0.13	0.11
Rough lemon	M	0.29	0.16	0.19	0.15
Sweet lime	S	0.14	0.19	0.15	0.14
Volkamer	S	0.21	0.14	0.36	0.16

Source of variation	df	Total Treatment Variation SS	Total Treatment Variation Percentage
Rootstock	5	0.22 **	37.29
Salinity	1	0.00 ns	0.00
Nematode	1	0.06 *	10.17
R x S	5	0.08 ns	13.56
R x N	5	0.13 †	22.03
S x N	1	0.02 ns	3.39
R x S x N	5	0.07 ns	11.86
Error	168	2.79	

**Significant at $P \leq 0.01$, *$P \leq 0.05$, † $P \leq 0.10$; ns = not significant at $P \leq 0.10$.

APPENDIX 15. *Tylenchulus semipenetrans* densities on sour orange split-roots with and without salinity.

Treatment	N	Nematode stages/g fresh roots		
		Females	Juveniles	Eggs
T/T	20	577bc	1,298b	13,419b
T/0	10	564c	1,768b	17,295b
T/S	10	999a	3,617a	31,157a
TS/0	10	724b	1,968b	18,805b
TS/TS	20	298c	1,009b	7,935c

Column means with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

APPENDIX 16. Chloride (Cl), sodium (Na), and potassium (K) as affected by combinations of Tylenchulus semipenetrans infection and salinity stress on sour orange split-roots.

Treatment	Cl	Na	K
0/0	0.12d	0.24d	1.61ab
S/0	0.61c	0.43c	1.54ab
T/0	0.22d	0.26d	1.33cd
T/S	0.52c	0.39c	1.42bc
TS/0	0.92b	0.34cd	1.81a
S/S	1.20b	0.52b	1.23cd
T/T	0.34d	0.47bc	0.91e
TS/TS	2.67a	0.81a	0.58f

Column means (n = 10) with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

APPENDIX 17. Plant growth as affected by a combination of salinity and *Tylenchulus semipenetrans* infection on sour orange with split-roots.

	Shoot		Root
Treatment	Weight (g)	Height (cm)	Weight (g)
0/0	18.37a	28.9a	3.28a
S/0	10.28bc	25.4bc	3.49a
T/0	11.72b	26.5ab	3.40a
T/S	10.41bc	25.6bc	2.94ab
TS/0	8.52c	22.6cd	2.86ab
S/S	11.58b	25.0bc	2.66b
T/T	9.07cd	21.3d	2.39bc
TS/TS	6.83d	19.0d	2.03c

Column means with the same letter are not different ($P \leq 0.05$) according to Duncan's multiple-range test.

APPENDIX 18. Nonosmoticum ions in leaves and roots of Cleopatra mandarin as affected by *Tylenchulus semipenetrans* and mechanical root pruning.

	Root	Macronutrients (%)			Micronutrients (ppm)			
Tissue	treatment	Ca	Mg	P	Cu	Fe	Mn	Zn
Leaf	Control	2.19a	0.31a	0.20a	11b	124b	36a	62a
	Nematode	2.14a	0.30a	0.19a	18b	106c	34ab	67a
	Pruned	1.93b	0.29a	0.16b	40a	137a	32b	73a
Root	Control	0.61b	0.23a	0.14b	30b	590a	126a	208a
	Nematode	0.60b	0.23a	0.15b	32b	531a	93b	159b
	Pruned	0.64a	0.22a	0.19a	178a	446b	49c	104c

Data pooled across salinity.

Column means with (n = 20) with the same letter are not different ($\underline{P} \leq 0.05$) according to Duncan's multiple-range test.

APPENDIX 19. Nonosmoticum ions in leaves and roots of sour orange as affected by *Tylenchulus semipenetrans* and mechanical root pruning.

	Root	Macronutrients (%)			Micronutrients (ppm)			
Tissue	treatment	Ca	Mg	P	Cu	Fe	Mn	Zn
Leaf	Control	1.44a	0.35a	0.24a	14a	101a	35a	59a
	Nematode	1.46a	0.34a	0.27a	13a	103a	34a	61a
	Pruned	1.30b	0.36a	0.29a	14a	99a	28b	60a
Root	Control	0.16b	0.33a	0.14b	30b	590a	126a	208a
	Nematode	0.60a	0.33a	0.15b	32b	531a	93b	159b
	Pruned	0.64a	0.34a	0.19a	170a	446b	49c	104c

Data pooled across salinity.

Column means with (n = 20) with the same letter are not different ($\underline{P} \leq 0.05$) according to Duncan's multiple-range test.

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BIOGRAPHICAL SKETCH

William Phatu Mashela entered the Master of Science (Nematology) program in August, 1987. Upon graduation in December, 1989, he proceeded to the Ph.D. program in Nematology (major) and Horticulture (minor). William is indigenous to South Africa, where he received BSc. Agric. (1984) and BSc. Agric. Honors (1986) degrees at the University of Fort Hare.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

L. W. Duncan, Chair
Associate Professor of
Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

R. McSorley, Cochair
Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. P. Syvertsen
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. H. Graham
Professor of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. H. O'Bannon
Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph W. Noling

J. W. Noling
Associate Professor of
Entomology and Nematology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1992

Jack L. Fry

Dean, College of
Agriculture

Dean, Graduate School